

# Supplementary Data

## Large-Scale Purification and Characterization of Recombinant Receptor-Binding Domain (RBD) of SARS-CoV-2 Spike Protein Expressed in Yeast

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Supplementary Table S1: Fermentation media

| Sr. No. | Name of the component     | Quantity (g/L) |
|---------|---------------------------|----------------|
| 1       | Yeast Extract             | 10             |
| 2       | Peptone                   | 20             |
| 3       | YNB                       | 13.4           |
| 4       | 1 M Phosphate buffer pH 6 | 100ml/L        |
| 5       | Glycerol                  | 10 ml/L        |
| 6       | Biotin 0.02%              | 4 ml/L         |

Supplementary Table S2: Fermentation dosing solutions

| Sr. No. | Name of the component                     | Remark                           |
|---------|---|----------------------------------|
| 1       | 50 % ammonia solution (alkali)            | for maintaining pH               |
| 2       | 10% Ortho Phosphoric Acid solution (acid) | for maintaining pH               |
| 3       | 10% antifoam solution                     | to prevent the formation of foam |
| 4       | 50% glycerol solution as substrate feed   | as substrate feed                |
| 5       | 50% sorbitol as induction feed            | as induction feed                |

Supplementary Table S3: Fermentation parameters

| Sr. No.                                    | Parameter            | Setpoint  | Mode   |
|--|----------------------|-----------|--|
| Shake Flask -1 parameter (100 mL)          |                      |           |  |
| 1  | Temperature          | 25 °C     | Controlled   |
| 2  | Agitation (RPM)      | 300       | Controlled   |
| 3  | Harvest OD at 600 nm | 5 to 15   | Process output   |
| Shake Flask -2 parameter (4 L)             |                      |           |  |
| 1  | Temperature          | 25 °C     | Controlled   |
| 2  | Agitation (RPM)      | 300       | Controlled   |
| 3  | Harvest OD at 600 nm | 5 to 15   | Process output   |
| Seed fermentation parameter (170 L)        |                      |           |  |
| 1  | Temperature          | 25 °C     | Controlled   |
| 2  | pH                   | 6.5 ± 0.1 | Controlled   |
| 3  | Aeration (SLPM)      | 75 -120   | Cascade mode with set point of DO 25% in controlled mode |
| 4  | OHP (bar)            | 0.2 – 1.0 |  |
| 5  | Agitation (RPM)      | 100 - 600 |  |
| 6  | Harvest OD at 600 nm | 25 to 30  | Process output   |
| Production fermentation parameter (1200 L) |                      |           |  |
| 1  | Temperature          | 25 °C     | Controlled   |

|   |                      |            |  |
|---|----------------------|------------|--|
| 2 | pH                   | 6.5 ± 0.1  | Controlled   |
| 3 | Aeration (SLPM)      | 450 to 550 | Cascade mode with set point of DO 25% in controlled mode |
| 4 | OHP (bar)            | 0.2 to 1   |  |
| 5 | Agitation (RPM)      | 56 to 210  |  |
| 6 | Harvest OD at 600 nm | ≥40 AU     | Process output   |

Supplementary Table S4: LC-MS-based peptide mapping

| Sample Batch No. | Retention Time (minutes) | Precursor m/z | Precursor Charge | Precursor Mass (Da) | Base Peptide Sequence         | Start Residue Number | Stop Residue Number | Missed Cleavages | Theoretical Mass (Da) | Precursor Mass Error (Da) | Precursor Mass Error (ppm) | Enzyme  |
|------------------|--------------------------|---------------|------------------|---------------------|-------------------------------|----------------------|---------------------|------------------|-----------------------|---------------------------|----------------------------|---------|
| 12681T002        | 30.89                    | 557.280       | 2                | 1112.545            | FASVYAWNRR                    | 16                   | 24                  | 0                | 1112.540              | 0.005                     | 4.398                      | Trypsin |
| 12681T002        | 27.24                    | 621.326       | 2                | 1240.637            | FASVYAWNRRK                   | 16                   | 25                  | 1                | 1240.635              | 0.002                     | 1.640                      | Trypsin |
| 12681T002        | 37.64                    | 699.364       | 2                | 1396.714            | FASVYAWNRRK                   | 16                   | 26                  | 2                | 1396.736              | -0.022                    | -15.845                    | Trypsin |
| 12681T002        | 18.29                    | 1329.142      | 2                | 2656.269            | KRISNCVADYSVLVNSASFSTFK       | 25                   | 47                  | 2                | 2656.296              | -0.026                    | -9.968                     | Trypsin |
| 12681T002        | 40.24                    | 1265.108      | 2                | 2528.202            | RISNCVADYSVLVNSASFSTFK        | 26                   | 47                  | 1                | 2528.201              | 0.002                     | 0.653                      | Trypsin |
| 12681T002        | 44.85                    | 1187.054      | 2                | 2372.093            | ISNCVADYSVLVNSASFSTFK         | 27                   | 47                  | 0                | 2372.099              | -0.006                    | -2.577                     | Trypsin |
| 12681T002        | 16.71                    | 456.219       | 2                | 910.423             | CYGVSPDK                      | 48                   | 55                  | 0                | 910.422               | 0.002                     | 1.688                      | Trypsin |
| 12681T002        | 49.48                    | 1024.001      | 2                | 2045.988            | LNDLCFTNVYADSFVIR             | 56                   | 72                  | 0                | 2045.988              | 0.000                     | 0.042                      | Trypsin |
| 12681T002        | 44.15                    | 1302.128      | 2                | 2602.241            | LNDLCFTNVYADSFVIRGDEVIR       | 56                   | 77                  | 1                | 2602.249              | -0.007                    | -2.754                     | Trypsin |
| 12681T002        | 5.56                     | 288.143       | 2                | 574.272             | GDEVIR                        | 73                   | 77                  | 0                | 574.271               | 0.001                     | 1.085                      | Trypsin |
| 12681T002        | 13.03                    | 450.252       | 2                | 898.490             | QIAPGQTGK                     | 78                   | 86                  | 0                | 898.487               | 0.003                     | 2.788                      | Trypsin |
| 12681T002        | 18.39                    | 443.720       | 2                | 885.425             | IADYNYK                       | 87                   | 93                  | 0                | 885.423               | 0.002                     | 2.557                      | Trypsin |
| 12681T002        | 43.68                    | 1567.229      | 2                | 3132.444            | IADYNYKLPPDFTGCVIAWNSNNLDSK   | 87                   | 113                 | 1                | 3132.450              | -0.006                    | -1.821                     | Trypsin |
| 12681T002        | 41.84                    | 1133.528      | 2                | 2265.042            | LPDDFTGCVIAWNSNNLDSK          | 94                   | 113                 | 0                | 2265.037              | 0.005                     | 2.215                      | Trypsin |
| 12681T002        | 27.45                    | 609.801       | 2                | 1217.588            | VGGNYNYLFR                    | 114                  | 123                 | 0                | 1217.583              | 0.005                     | 4.250                      | Trypsin |
| 12681T002        | 40.60                    | 817.925       | 2                | 1633.836            | VGGNYNYLRLFR                  | 114                  | 126                 | 1                | 1633.837              | 0.000                     | -0.134                     | Trypsin |
| 12681T002        | 50.31                    | 881.972       | 2                | 1761.930            | VGGNYNYLRLFRK                 | 114                  | 127                 | 2                | 1761.931              | -0.002                    | -1.052                     | Trypsin |
| 12681T002        | 13.22                    | 1367.709      | 2                | 2733.404            | VGGNYNYLRLFRKSNLKPFR          | 114                  | 135                 | 3                | 2733.450              | -0.046                    | -16.797                    | Trypsin |
| 12681T002        | 11.10                    | 282.187       | 2                | 562.360             | LFRK                          | 124                  | 127                 | 1                | 562.359               | 0.001                     | 1.193                      | Trypsin |
| 12681T002        | 16.75                    | 218.139       | 2                | 434.263             | LFR                           | 124                  | 126                 | 0                | 434.264               | -0.001                    | -1.608                     | Trypsin |
| 12681T002        | 16.40                    | 559.822       | 2                | 1117.629            | KSNLKPFR                      | 127                  | 135                 | 1                | 1117.624              | 0.004                     | 3.928                      | Trypsin |
| 12681T002        | 19.44                    | 495.774       | 2                | 989.533             | SNLKPFR                       | 128                  | 135                 | 0                | 989.529               | 0.003                     | 3.262                      | Trypsin |
| 12681T002        | 43.61                    | 1019.069      | 2                | 2036.123            | VVLSFELLHAPATVCGPK            | 179                  | 197                 | 0                | 2036.113              | 0.010                     | 5.008                      | Trypsin |
| 12681T002        | 40.43                    | 1083.111      | 2                | 2164.208            | VVLSFELLHAPATVCGPKK           | 179                  | 198                 | 1                | 2164.208              | 0.000                     | 0.225                      | Trypsin |
| 12681T002        | 38.61                    | 1352.617      | 2                | 2703.219            | KSTNGGDDGGDGGGAHIVMVDAYKPTK   | 198                  | 225                 | 1                | 2703.256              | -0.036                    | -13.468                    | Trypsin |
| 12681T002        | 27.55                    | 1288.589      | 2                | 2575.164            | STNGGDDGGDGGGAHIVMVDAYKPTK    | 199                  | 225                 | 0                | 2575.161              | 0.003                     | 1.024                      | Trypsin |
| 12681T002        | 24.97                    | 1296.586      | 2                | 2591.157            | STNGGDDGGDGGGAHIVMVDAYKPTK    | 199                  | 225                 | 0                | 2591.156              | 0.001                     | 0.575                      | Trypsin |
| 12681T002        | 36.17                    | 525.751       | 2                | 1049.488            | ITNLCPFE                      | 1                    | 9                   | 0                | 1049.485              | 0.003                     | 2.619                      | Glu-C   |
| 12681T002        | 22.80                    | 698.316       | 2                | 1394.617            | IYQAGSTPCNGVE                 | 141                  | 153                 | 0                | 1394.614              | 0.004                     | 2.568                      | Glu-C   |
| 12681T003        | 32.00                    | 557.280       | 2                | 1112.545            | FASVYAWNRR                    | 16                   | 24                  | 0                | 1112.540              | 0.005                     | 4.189                      | Trypsin |
| 12681T003        | 27.79                    | 621.327       | 2                | 1240.638            | FASVYAWNRRK                   | 16                   | 25                  | 1                | 1240.635              | 0.003                     | 2.584                      | Trypsin |
| 12681T003        | 18.39                    | 1329.144      | 2                | 2656.273            | KRISNCVADYSVLVNSASFSTFK       | 25                   | 47                  | 2                | 2656.296              | -0.023                    | -8.626                     | Trypsin |
| 12681T003        | 39.98                    | 1265.108      | 2                | 2528.202            | RISNCVADYSVLVNSASFSTFK        | 26                   | 47                  | 1                | 2528.201              | 0.001                     | 0.580                      | Trypsin |
| 12681T003        | 44.36                    | 1187.056      | 2                | 2372.097            | ISNCVADYSVLVNSASFSTFK         | 27                   | 47                  | 0                | 2372.099              | -0.002                    | -0.870                     | Trypsin |
| 12681T003        | 42.13                    | 1633.266      | 2                | 3264.517            | ISNCVADYSVLVNSASFSTFKCYGVSPDK | 27                   | 55                  | 1                | 3264.511              | 0.006                     | 1.953                      | Trypsin |
| 12681T003        | 16.40                    | 456.219       | 2                | 910.424             | CYGVSPDK                      | 48                   | 55                  | 0                | 910.422               | 0.002                     | 2.469                      | Trypsin |
| 12681T003        | 46.75                    | 1024.002      | 2                | 2045.989            | LNDLCFTNVYADSFVIR             | 56                   | 72                  | 0                | 2045.988              | 0.001                     | 0.516                      | Trypsin |
| 12681T003        | 43.86                    | 1302.133      | 2                | 2602.252            | LNDLCFTNVYADSFVIRGDEVIR       | 56                   | 77                  | 1                | 2602.249              | 0.003                     | 1.328                      | Trypsin |
| 12681T003        | 17.79                    | 728.379       | 2                | 1454.743            | GDEVIRQIAPGQTGK               | 73                   | 86                  | 1                | 1454.748              | -0.005                    | -3.428                     | Trypsin |
| 12681T003        | 5.63                     | 288.143       | 2                | 574.271             | GDEVIR                        | 73                   | 77                  | 0                | 574.271               | 0.000                     | 0.243                      | Trypsin |
| 12681T003        | 12.57                    | 450.254       | 2                | 898.493             | QIAPGQTGK                     | 78                   | 86                  | 0                | 898.487               | 0.006                     | 6.486                      | Trypsin |
| 12681T003        | 49.79                    | 883.945       | 2                | 1765.875            | QIAPGQTGKIADYNYK              | 78                   | 93                  | 1                | 1765.900              | -0.025                    | -13.937                    | Trypsin |
| 12681T003        | 43.64                    | 1567.232      | 2                | 3132.450            | IADYNYKLPPDFTGCVIAWNSNNLDSK   | 87                   | 113                 | 1                | 3132.450              | 0.000                     | 0.026                      | Trypsin |
| 12681T003        | 18.48                    | 443.721       | 2                | 885.427             | IADYNYK                       | 87                   | 93                  | 0                | 885.423               | 0.004                     | 4.396                      | Trypsin |
| 12681T003        | 41.64                    | 1133.530      | 2                | 2265.046            | LPDDFTGCVIAWNSNNLDSK          | 94                   | 113                 | 0                | 2265.037              | 0.009                     | 3.880                      | Trypsin |

|           |       |          |   |          |                              |     |     |   |          |        |         |         |
|-----------|-------|----------|---|----------|------------------------------|-----|-----|---|----------|--------|---------|---------|
| 12681T003 | 27.79 | 609.802  | 2 | 1217.590 | VGGNYNYLYR                   | 114 | 123 | 0 | 1217.583 | 0.007  | 6.003   | Trypsin |
| 12681T003 | 50.36 | 881.974  | 2 | 1761.933 | VGGNYNYLYRFRK                | 114 | 127 | 2 | 1761.931 | 0.002  | 1.095   | Trypsin |
| 12681T003 | 40.56 | 817.927  | 2 | 1633.838 | VGGNYNYLYRFLR                | 114 | 126 | 1 | 1633.837 | 0.002  | 1.195   | Trypsin |
| 12681T003 | 12.74 | 1367.724 | 2 | 2733.433 | VGGNYNYLYRFRKSNLKPFR         | 114 | 135 | 3 | 2733.450 | -0.018 | -6.470  | Trypsin |
| 12681T003 | 10.83 | 282.188  | 2 | 562.362  | LFRK                         | 124 | 127 | 1 | 562.359  | 0.003  | 5.755   | Trypsin |
| 12681T003 | 15.93 | 218.140  | 2 | 434.266  | LFR                          | 124 | 126 | 0 | 434.264  | 0.002  | 4.688   | Trypsin |
| 12681T003 | 15.93 | 559.822  | 2 | 1117.630 | KSNLKPFR                     | 127 | 135 | 1 | 1117.624 | 0.006  | 4.966   | Trypsin |
| 12681T003 | 19.33 | 495.775  | 2 | 989.535  | SNLKPFR                      | 128 | 135 | 0 | 989.529  | 0.005  | 5.528   | Trypsin |
| 12681T003 | 43.27 | 1019.072 | 2 | 2036.129 | VVLSFELLHAPATVCGPK           | 179 | 197 | 0 | 2036.113 | 0.016  | 7.790   | Trypsin |
| 12681T003 | 40.17 | 1083.115 | 2 | 2164.215 | VVLSFELLHAPATVCGPKK          | 179 | 198 | 1 | 2164.208 | 0.007  | 3.272   | Trypsin |
| 12681T003 | 28.21 | 1288.586 | 2 | 2575.158 | STNGGDDGGDGGGAHVMVDAYKPTK    | 199 | 225 | 0 | 2575.161 | -0.003 | -1.004  | Trypsin |
| 12681T003 | 25.08 | 1296.587 | 2 | 2591.159 | STNGGDDGGDGGGAHVMVDAYKPTK    | 199 | 225 | 0 | 2591.156 | 0.003  | 1.184   | Trypsin |
| 12681T003 | 34.57 | 525.753  | 2 | 1049.491 | ITNLCPFGE                    | 1   | 9   | 0 | 1049.485 | 0.006  | 5.895   | Glu-C   |
| 12681T003 | 22.67 | 698.315  | 2 | 1394.615 | IYQAGSTPCNGVE                | 141 | 153 | 0 | 1394.614 | 0.001  | 0.738   | Glu-C   |
| 12681T004 | 31.58 | 557.279  | 2 | 1112.544 | FASVYAWNR                    | 16  | 24  | 0 | 1112.540 | 0.003  | 3.054   | Trypsin |
| 12681T004 | 27.09 | 621.325  | 2 | 1240.635 | FASVYAWNRK                   | 16  | 25  | 1 | 1240.635 | -0.001 | -0.440  | Trypsin |
| 12681T004 | 38.00 | 699.362  | 2 | 1396.709 | FASVYAWNRKR                  | 16  | 26  | 2 | 1396.736 | -0.027 | -19.468 | Trypsin |
| 12681T004 | 18.38 | 1329.149 | 2 | 2656.283 | KRISNCVADYSVLNSASFSTFK       | 25  | 47  | 2 | 2656.296 | -0.013 | -4.765  | Trypsin |
| 12681T004 | 40.14 | 1265.107 | 2 | 2528.200 | RISNCVADYSVLNSASFSTFK        | 26  | 47  | 1 | 2528.201 | 0.000  | -0.117  | Trypsin |
| 12681T004 | 42.26 | 1187.059 | 2 | 2372.103 | ISNCVADYSVLNSASFSTFK         | 27  | 47  | 0 | 2372.099 | 0.003  | 1.409   | Trypsin |
| 12681T004 | 41.55 | 1633.262 | 2 | 3264.510 | ISNCVADYSVLNSASFSTFKCYGVSPTK | 27  | 55  | 1 | 3264.511 | 0.000  | -0.136  | Trypsin |
| 12681T004 | 16.61 | 456.218  | 2 | 910.422  | CYGVSPTK                     | 48  | 55  | 0 | 910.422  | 0.000  | 0.153   | Trypsin |
| 12681T004 | 46.18 | 1024.003 | 2 | 2045.992 | LNDLCFTNVYADSFVIR            | 56  | 72  | 0 | 2045.988 | 0.004  | 1.783   | Trypsin |
| 12681T004 | 43.49 | 1302.132 | 2 | 2602.250 | LNDLCFTNVYADSFVIRGDEVR       | 56  | 77  | 1 | 2602.249 | 0.001  | 0.537   | Trypsin |
| 12681T004 | 5.80  | 288.143  | 2 | 574.271  | GDEVR                        | 73  | 77  | 0 | 574.271  | 0.000  | 0.269   | Trypsin |
| 12681T004 | 17.83 | 728.384  | 2 | 1454.754 | GDEVRQIAPGQTGK               | 73  | 86  | 1 | 1454.748 | 0.006  | 4.306   | Trypsin |
| 12681T004 | 13.23 | 450.252  | 2 | 898.490  | QIAPGQTGK                    | 78  | 86  | 0 | 898.487  | 0.003  | 3.172   | Trypsin |
| 12681T004 | 49.60 | 883.942  | 2 | 1765.870 | QIAPGQTGKIADYNYK             | 78  | 93  | 1 | 1765.900 | -0.030 | -16.999 | Trypsin |
| 12681T004 | 42.94 | 1567.228 | 2 | 3132.442 | IADYNYKLPDDFTGCVIAWNSNNLDSK  | 87  | 113 | 1 | 3132.450 | -0.008 | -2.427  | Trypsin |
| 12681T004 | 18.48 | 443.722  | 2 | 885.429  | IADYNYK                      | 87  | 93  | 0 | 885.423  | 0.006  | 6.709   | Trypsin |
| 12681T004 | 41.37 | 1133.528 | 2 | 2265.042 | LPDDFTGCVIAWNSNNLDSK         | 94  | 113 | 0 | 2265.037 | 0.005  | 2.071   | Trypsin |
| 12681T004 | 27.34 | 609.802  | 2 | 1217.589 | VGGNYNYLYR                   | 114 | 123 | 0 | 1217.583 | 0.006  | 4.906   | Trypsin |
| 12681T004 | 13.20 | 1367.717 | 2 | 2733.420 | VGGNYNYLYRFRKSNLKPFR         | 114 | 135 | 3 | 2733.450 | -0.030 | -11.010 | Trypsin |
| 12681T004 | 11.81 | 282.188  | 2 | 562.362  | LFRK                         | 124 | 127 | 1 | 562.359  | 0.003  | 5.864   | Trypsin |
| 12681T004 | 16.08 | 218.140  | 2 | 434.266  | LFR                          | 124 | 126 | 0 | 434.264  | 0.002  | 3.640   | Trypsin |
| 12681T004 | 16.05 | 559.821  | 2 | 1117.627 | KSNLKPFR                     | 127 | 135 | 1 | 1117.624 | 0.003  | 2.633   | Trypsin |
| 12681T004 | 19.55 | 495.774  | 2 | 989.534  | SNLKPFR                      | 128 | 135 | 0 | 989.529  | 0.004  | 4.258   | Trypsin |
| 12681T004 | 42.54 | 1019.068 | 2 | 2036.121 | VVLSFELLHAPATVCGPK           | 179 | 197 | 0 | 2036.113 | 0.008  | 4.003   | Trypsin |
| 12681T004 | 40.14 | 1083.112 | 2 | 2164.210 | VVLSFELLHAPATVCGPKK          | 179 | 198 | 1 | 2164.208 | 0.002  | 0.902   | Trypsin |
| 12681T004 | 27.85 | 1288.586 | 2 | 2575.158 | STNGGDDGGDGGGAHVMVDAYKPTK    | 199 | 225 | 0 | 2575.161 | -0.003 | -1.203  | Trypsin |
| 12681T004 | 36.11 | 525.751  | 2 | 1049.487 | ITNLCPFGE                    | 1   | 9   | 0 | 1049.485 | 0.002  | 1.591   | Glu-C   |
| 12681T004 | 22.72 | 698.318  | 2 | 1394.622 | IYQAGSTPCNGVE                | 141 | 153 | 0 | 1394.614 | 0.008  | 5.750   | Glu-C   |
| 12681T005 | 32.43 | 557.281  | 2 | 1112.548 | FASVYAWNR                    | 16  | 24  | 0 | 1112.540 | 0.007  | 6.601   | Trypsin |
| 12681T005 | 28.01 | 621.325  | 2 | 1240.635 | FASVYAWNRK                   | 16  | 25  | 1 | 1240.635 | -0.001 | -0.551  | Trypsin |
| 12681T005 | 18.59 | 1329.137 | 2 | 2656.259 | KRISNCVADYSVLNSASFSTFK       | 25  | 47  | 2 | 2656.296 | -0.036 | -13.574 | Trypsin |
| 12681T005 | 41.82 | 1265.110 | 2 | 2528.206 | RISNCVADYSVLNSASFSTFK        | 26  | 47  | 1 | 2528.201 | 0.005  | 2.013   | Trypsin |
| 12681T005 | 45.36 | 1187.057 | 2 | 2372.100 | ISNCVADYSVLNSASFSTFK         | 27  | 47  | 0 | 2372.099 | 0.000  | 0.176   | Trypsin |
| 12681T005 | 43.62 | 1633.260 | 2 | 3264.506 | ISNCVADYSVLNSASFSTFKCYGVSPTK | 27  | 55  | 1 | 3264.511 | -0.005 | -1.552  | Trypsin |

|           |       |          |   |          |                             |     |     |   |          |        |         |         |
|-----------|-------|----------|---|----------|-----------------------------|-----|-----|---|----------|--------|---------|---------|
| 12681T005 | 16.75 | 456.216  | 2 | 910.418  | CYGVSPTK                    | 48  | 55  | 0 | 910.422  | -0.004 | -4.501  | Trypsin |
| 12681T005 | 49.90 | 1024.004 | 2 | 2045.993 | LNDLCFTNVYADSFVIR           | 56  | 72  | 0 | 2045.988 | 0.005  | 2.268   | Trypsin |
| 12681T005 | 45.24 | 1302.132 | 2 | 2602.249 | LNDLCFTNVYADSFVIRGDEVR      | 56  | 77  | 1 | 2602.249 | 0.000  | 0.061   | Trypsin |
| 12681T005 | 18.12 | 728.382  | 2 | 1454.749 | GDEVRQIAPGQTGK              | 73  | 86  | 1 | 1454.748 | 0.001  | 0.890   | Trypsin |
| 12681T005 | 12.22 | 450.253  | 2 | 898.491  | QIAPGQTGK                   | 78  | 86  | 0 | 898.487  | 0.004  | 4.377   | Trypsin |
| 12681T005 | 51.43 | 883.946  | 2 | 1765.878 | QIAPGQTGKIADYNYK            | 78  | 93  | 1 | 1765.900 | -0.022 | -12.236 | Trypsin |
| 12681T005 | 44.71 | 1567.235 | 2 | 3132.455 | IADYNYKLPDDFTGCVIAWNSNNLDSK | 87  | 113 | 1 | 3132.450 | 0.005  | 1.603   | Trypsin |
| 12681T005 | 18.69 | 443.721  | 2 | 885.428  | IADYNYK                     | 87  | 93  | 0 | 885.423  | 0.005  | 5.456   | Trypsin |
| 12681T005 | 43.27 | 1133.530 | 2 | 2265.046 | LPDDFTGCVIAWNSNNLDSK        | 94  | 113 | 0 | 2265.037 | 0.009  | 3.889   | Trypsin |
| 12681T005 | 28.03 | 609.802  | 2 | 1217.590 | VGGNYNYLYR                  | 114 | 123 | 0 | 1217.583 | 0.007  | 5.757   | Trypsin |
| 12681T005 | 9.80  | 282.189  | 2 | 562.362  | LFRK                        | 124 | 127 | 1 | 562.359  | 0.003  | 5.923   | Trypsin |
| 12681T005 | 15.59 | 218.141  | 2 | 434.267  | LFR                         | 124 | 126 | 0 | 434.264  | 0.003  | 5.941   | Trypsin |
| 12681T005 | 16.22 | 559.823  | 2 | 1117.632 | KSNLKPFR                    | 127 | 135 | 1 | 1117.624 | 0.008  | 7.016   | Trypsin |
| 12681T005 | 19.60 | 495.776  | 2 | 989.538  | SNLKPFR                     | 128 | 135 | 0 | 989.529  | 0.009  | 8.966   | Trypsin |
| 12681T005 | 44.45 | 1019.071 | 2 | 2036.128 | VVLSFELLHAPATVCGPK          | 179 | 197 | 0 | 2036.113 | 0.015  | 7.262   | Trypsin |
| 12681T005 | 42.06 | 1083.113 | 2 | 2164.211 | VVLSFELLHAPATVCGPKK         | 179 | 198 | 1 | 2164.208 | 0.003  | 1.515   | Trypsin |
| 12681T005 | 28.83 | 1288.590 | 2 | 2575.165 | STNGGDDGGDGGGAHVMVDAYKPTK   | 199 | 225 | 0 | 2575.161 | 0.004  | 1.663   | Trypsin |
| 12681T005 | 35.46 | 525.749  | 2 | 1049.483 | ITNLCPFGE                   | 1   | 9   | 0 | 1049.485 | -0.002 | -1.931  | Glu-C   |
| 12681T005 | 22.98 | 698.316  | 2 | 1394.618 | IYQAGSTPCNGVE               | 141 | 153 | 0 | 1394.614 | 0.004  | 2.788   | Glu-C   |



Supplementary Table S5: Identified Glycans from Sample Batch No. 12681T002

| Glycoform Mass | Glycan Structure   | Glycan Type    | Peptide Mass [M] | Peptide Sequence | Theoretical Glycopeptide Mass | $\Delta$ mass (Dalton) |
|----------------|--|----------------|------------------|------------------|-------------------------------|------------------------|
| 527.185        | (Hex) <sub>2</sub> (HexNAc) <sub>1</sub>   | -              | 1737.851         | ITNLCPFGEVFNATR  | 2266.043                      | 0                      |
| 656.228        | (Hex) <sub>1</sub> (HexNAc) <sub>1</sub> (NeuAc) <sub>1</sub>  | -              | 1680.829         | ITNLCPFGEVFNATR  | 2338.065                      | -0.001                 |
| 846.312        | (Hex) <sub>1</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>1</sub> (Pent) <sub>1</sub>                        | -              | 1680.829         | ITNLCPFGEVFNATR  | 2528.149                      | 0                      |
| 851.291        | (Hex) <sub>4</sub> (HexNAc) <sub>1</sub>   | -              | 1737.851         | ITNLCPFGEVFNATR  | 2590.149                      | 0.002                  |
| 1008.365       | (Hex) <sub>2</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>1</sub> (Pent) <sub>1</sub>                        | -              | 1737.851         | ITNLCPFGEVFNATR  | 2747.223                      | 0                      |
| 1170.417       | (Hex) <sub>3</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>1</sub> (Pent) <sub>1</sub>                        | -              | 1680.829         | ITNLCPFGEVFNATR  | 2852.254                      | 0                      |
| 1175.396       | (Hex) <sub>6</sub> (HexNAc) <sub>1</sub>   | -              | 1737.851         | ITNLCPFGEVFNATR  | 2914.254                      | 0.002                  |
| 1332.47        | (Hex) <sub>4</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>1</sub> (Pent) <sub>1</sub>                        | -              | 1680.829         | ITNLCPFGEVFNATR  | 3014.307                      | 0                      |
| 1346.486       | (Hex) <sub>4</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>2</sub>  | -              | 1737.851         | ITNLCPFGEVFNATR  | 3085.344                      | 0                      |
| 1387.512       | (HexNAc) <sub>1</sub> (Deoxyhexose) <sub>2</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>                    | hybrid/complex | 1680.829         | ITNLCPFGEVFNATR  | 3069.349                      | 0.005                  |
| 1403.507       | (Hex) <sub>1</sub> (HexNAc) <sub>1</sub> (Deoxyhexose) <sub>1</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub> | hybrid/complex | 1680.829         | ITNLCPFGEVFNATR  | 3085.344                      | 0.001                  |
| 1403.507       | (Hex) <sub>1</sub> (HexNAc) <sub>1</sub> (Deoxyhexose) <sub>1</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub> | hybrid/complex | 1737.851         | ITNLCPFGEVFNATR  | 3142.365                      | 0.005                  |
| 1460.529       | (Hex) <sub>1</sub> (HexNAc) <sub>2</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>                            | hybrid/complex | 1680.829         | ITNLCPFGEVFNATR  | 3142.366                      | 0.004                  |
| 2076.75        | (Hex) <sub>3</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>2</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub> | hybrid/complex | 1680.829         | ITNLCPFGEVFNATR  | 3758.587                      | 0.005                  |

Supplementary Table S6: Identified Glycans from Sample Batch No. 12681T003

| Glycoform Mass | Glycan Structure  | Glycan Type    | Peptide Mass [M] | Peptide Sequence | Theoretical Glycopeptide Mass | $\Delta$ mass (Dalton) |
|----------------|---|----------------|------------------|------------------|-------------------------------|------------------------|
| 527.185        | (Hex) <sub>2</sub> (HexNAc) <sub>1</sub>  | -              | 1737.851         | ITNLCPFGGEVFNATR | 2266.043                      | 0                      |
| 656.228        | (Hex) <sub>1</sub> (HexNAc) <sub>1</sub> (NeuAc) <sub>1</sub>                           | -              | 1680.829         | ITNLCPFGGEVFNATR | 2338.065                      | -0.001                 |
| 730.264        | (Hex) <sub>2</sub> (HexNAc) <sub>2</sub>  | -              | 1737.851         | ITNLCPFGGEVFNATR | 2469.122                      | 0.003                  |
| 846.312        | (Hex) <sub>1</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>1</sub> (Pent) <sub>1</sub> | -              | 1737.851         | ITNLCPFGGEVFNATR | 2585.17                       | 0.001                  |
| 972.284        | (Hex) <sub>3</sub> (HexNAc) <sub>2</sub> (Phos) <sub>1</sub>                            | -              | 1737.851         | ITNLCPFGGEVFNATR | 2711.142                      | 0.004                  |
| 1008.365       | (Hex) <sub>2</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>1</sub> (Pent) <sub>1</sub> | -              | 1680.829         | ITNLCPFGGEVFNATR | 2690.202                      | 0.001                  |
| 1022.38        | (Hex) <sub>2</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>2</sub>                     | -              | 1680.829         | ITNLCPFGGEVFNATR | 2704.217                      | 0.003                  |
| 1170.417       | (Hex) <sub>3</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>1</sub> (Pent) <sub>1</sub> | -              | 1680.829         | ITNLCPFGGEVFNATR | 2852.254                      | 0.002                  |
| 1175.396       | (Hex) <sub>6</sub> (HexNAc) <sub>1</sub>  | -              | 1737.851         | ITNLCPFGGEVFNATR | 2914.254                      | 0.003                  |
| 1186.412       | (Hex) <sub>4</sub> (HexNAc) <sub>2</sub> (Pent) <sub>1</sub>                            | -              | 1737.851         | ITNLCPFGGEVFNATR | 2925.27                       | 0                      |
| 1332.47        | (Hex) <sub>4</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>1</sub> (Pent) <sub>1</sub> | -              | 1680.829         | ITNLCPFGGEVFNATR | 3014.307                      | 0.003                  |
| 1346.486       | (Hex) <sub>4</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>2</sub>                     | -              | 1737.851         | ITNLCPFGGEVFNATR | 3085.344                      | 0.004                  |
| 1387.512       | (HexNAc) <sub>1</sub> (Deoxyhexose) <sub>2</sub> +                                      | hybrid/complex | 1737.851         | ITNLCPFGGEVFNATR | 3126.37                       | 0.003                  |
| 1403.507       | (Hex) <sub>1</sub> (HexNAc) <sub>1</sub> (Deoxyhexose) <sub>1</sub> +                   | hybrid/complex | 1680.829         | ITNLCPFGGEVFNATR | 3085.344                      | 0.005                  |
| 1444.534       | (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>1</sub> +                                      | hybrid/complex | 1680.829         | ITNLCPFGGEVFNATR | 3126.371                      | 0.002                  |
| 1581.555       | (Hex) <sub>3</sub> (HexNAc) <sub>1</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>     | hybrid/complex | 1737.851         | ITNLCPFGGEVFNATR | 3320.413                      | 0                      |
| 1752.645       | (Hex) <sub>1</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>2</sub> +                   | hybrid/complex | 1737.851         | ITNLCPFGGEVFNATR | 3491.503                      | -0.002                 |
| 1793.671       | (HexNAc) <sub>3</sub> (Deoxyhexose) <sub>2</sub> +                                      | hybrid/complex | 1737.851         | ITNLCPFGGEVFNATR | 3532.529                      | 0                      |
| 1809.666       | (Hex) <sub>1</sub> (HexNAc) <sub>3</sub> (Deoxyhexose) <sub>1</sub> +                   | hybrid/complex | 1680.829         | ITNLCPFGGEVFNATR | 3491.503                      | -0.002                 |
| 1850.693       | (HexNAc) <sub>4</sub> (Deoxyhexose) <sub>1</sub> +                                      | hybrid/complex | 1680.829         | ITNLCPFGGEVFNATR | 3532.53                       | 0                      |
| 2028.74        | (Hex) <sub>2</sub> (HexNAc) <sub>4</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>     | hybrid/complex | 1737.851         | ITNLCPFGGEVFNATR | 3767.598                      | 0.005                  |
| 2076.75        | (Hex) <sub>3</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>2</sub> +                   | hybrid/complex | 1680.829         | ITNLCPFGGEVFNATR | 3758.587                      | 0.003                  |
| 2204.772       | (Hex) <sub>2</sub> (HexNAc) <sub>2</sub> (NeuAc) <sub>2</sub> +                         | hybrid/complex | 1737.851         | ITNLCPFGGEVFNATR | 3943.63                       | -0.002                 |
| 2205.793       | (Hex) <sub>2</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>2</sub>                     | hybrid/complex | 1737.851         | ITNLCPFGGEVFNATR | 3944.651                      | -0.003                 |
| 2238.803       | (Hex) <sub>4</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>2</sub> +                   | hybrid/complex | 1680.829         | ITNLCPFGGEVFNATR | 3920.64                       | 0.002                  |
| 2262.814       | (Hex) <sub>2</sub> (HexNAc) <sub>3</sub> (Deoxyhexose) <sub>1</sub>                     | hybrid/complex | 1680.829         | ITNLCPFGGEVFNATR | 3944.651                      | -0.002                 |
| 2465.894       | (Hex) <sub>2</sub> (HexNAc) <sub>4</sub> (Deoxyhexose) <sub>1</sub>                     | hybrid/complex | 1737.851         | ITNLCPFGGEVFNATR | 4204.752                      | -0.003                 |
| 2522.915       | (Hex) <sub>2</sub> (HexNAc) <sub>5</sub> (NeuAc) <sub>1</sub> +                         | hybrid/complex | 1680.829         | ITNLCPFGGEVFNATR | 4204.751                      | -0.002                 |
| 2586.92        | (Hex) <sub>4</sub> (HexNAc) <sub>3</sub> (Deoxyhexose) <sub>1</sub>                     | hybrid/complex | 1680.829         | ITNLCPFGGEVFNATR | 4268.757                      | 0.001                  |
| 3181.184       | (Hex) <sub>3</sub> (HexNAc) <sub>6</sub> (Deoxyhexose) <sub>4</sub> +                   | hybrid/complex | 1680.829         | ITNLCPFGGEVFNATR | 4863.021                      | 0                      |

Supplementary Table S7: Identified Glycans from Sample Batch No. 12681T004

| Glycoform Mass | Glycan Structure  | Glycan Type    | Peptide Mass [M] | Peptide Sequence | Theoretical Glycopeptide Mass | Δmass (Dalton) |
|----------------|---|----------------|------------------|------------------|-------------------------------|----------------|
| 527.185        | (Hex) <sub>2</sub> (HexNAc) <sub>1</sub>  | -              | 1737.851         | ITNLCPFGEVFNATR  | 2266.043                      | 0              |
| 656.228        | (Hex) <sub>1</sub> (HexNAc) <sub>1</sub> (NeuAc) <sub>1</sub>   | -              | 1737.851         | ITNLCPFGEVFNATR  | 2395.086                      | -0.003         |
| 846.312        | (Hex) <sub>1</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>1</sub> (Pent) <sub>1</sub>   | -              | 1737.851         | ITNLCPFGEVFNATR  | 2585.17                       | 0              |
| 1008.365       | (Hex) <sub>2</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>1</sub> (Pent) <sub>1</sub>   | -              | 1680.829         | ITNLCPFGEVFNATR  | 2690.202                      | -0.001         |
| 1013.343       | (Hex) <sub>5</sub> (HexNAc) <sub>1</sub>  | -              | 1680.829         | ITNLCPFGEVFNATR  | 2695.18                       | 0              |
| 1022.38        | (Hex) <sub>2</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>2</sub>   | -              | 1680.829         | ITNLCPFGEVFNATR  | 2704.217                      | 0.002          |
| 1170.417       | (Hex) <sub>3</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>1</sub> (Pent) <sub>1</sub>   | -              | 1680.829         | ITNLCPFGEVFNATR  | 2852.254                      | 0.005          |
| 1216.423       | (Hex) <sub>2</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>   | high_man       | 1737.851         | ITNLCPFGEVFNATR  | 2955.281                      | -0.002         |
| 1332.47        | (Hex) <sub>4</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>1</sub> (Pent) <sub>1</sub>   | -              | 1680.829         | ITNLCPFGEVFNATR  | 3014.307                      | -0.003         |
| 1378.476       | (Hex) <sub>3</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>   | high_man       | 1737.851         | ITNLCPFGEVFNATR  | 3117.334                      | 0.003          |
| 1387.512       | (HexNAc) <sub>1</sub> (Deoxyhexose) <sub>2</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>   | hybrid/complex | 1680.829         | ITNLCPFGEVFNATR  | 3069.349                      | 0.003          |
| 1540.528       | (Hex) <sub>4</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>   | high_man       | 1737.851         | ITNLCPFGEVFNATR  | 3279.386                      | 0.003          |
| 1651.597       | (HexNAc) <sub>1</sub> (Deoxyhexose) <sub>2</sub> (Pent) <sub>2</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>                     | hybrid/complex | 1680.829         | ITNLCPFGEVFNATR  | 3333.434                      | -0.004         |
| 1898.703       | (Hex) <sub>1</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>3</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>                      | hybrid/complex | 1737.851         | ITNLCPFGEVFNATR  | 3637.561                      | 0.001          |
| 1955.724       | (Hex) <sub>1</sub> (HexNAc) <sub>3</sub> (Deoxyhexose) <sub>2</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>                      | hybrid/complex | 1680.829         | ITNLCPFGEVFNATR  | 3637.561                      | 0.002          |
| 1971.719       | (Hex) <sub>2</sub> (HexNAc) <sub>3</sub> (Deoxyhexose) <sub>1</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>                      | hybrid/complex | 1737.851         | ITNLCPFGEVFNATR  | 3710.577                      | 0              |
| 2026.687       | (Hex) <sub>7</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>   | high_man       | 1737.851         | ITNLCPFGEVFNATR  | 3765.545                      | -0.004         |
| 2028.74        | (Hex) <sub>2</sub> (HexNAc) <sub>4</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>   | hybrid/complex | 1680.829         | ITNLCPFGEVFNATR  | 3710.577                      | 0              |
| 2035.681       | (Hex) <sub>1</sub> (HexNAc) <sub>3</sub> (Deoxyhexose) <sub>2</sub> (Sulph) <sub>1</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub> | hybrid/complex | 1737.851         | ITNLCPFGEVFNATR  | 3774.539                      | 0              |
| 2076.75        | (Hex) <sub>3</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>2</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>                      | hybrid/complex | 1680.829         | ITNLCPFGEVFNATR  | 3758.587                      | 0              |
| 2188.74        | (Hex) <sub>8</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>   | high_man       | 1737.851         | ITNLCPFGEVFNATR  | 3927.598                      | -0.003         |
| 2238.803       | (Hex) <sub>4</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>2</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>                      | hybrid/complex | 1680.829         | ITNLCPFGEVFNATR  | 3920.64                       | 0.005          |
| 2238.803       | (Hex) <sub>4</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>2</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>                      | hybrid/complex | 1737.851         | ITNLCPFGEVFNATR  | 3977.661                      | 0              |
| 2295.825       | (Hex) <sub>4</sub> (HexNAc) <sub>3</sub> (Deoxyhexose) <sub>1</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>                      | hybrid/complex | 1680.829         | ITNLCPFGEVFNATR  | 3977.662                      | -0.001         |



Supplementary Table S8: Identified Glycans from Sample Batch No. 12681T005

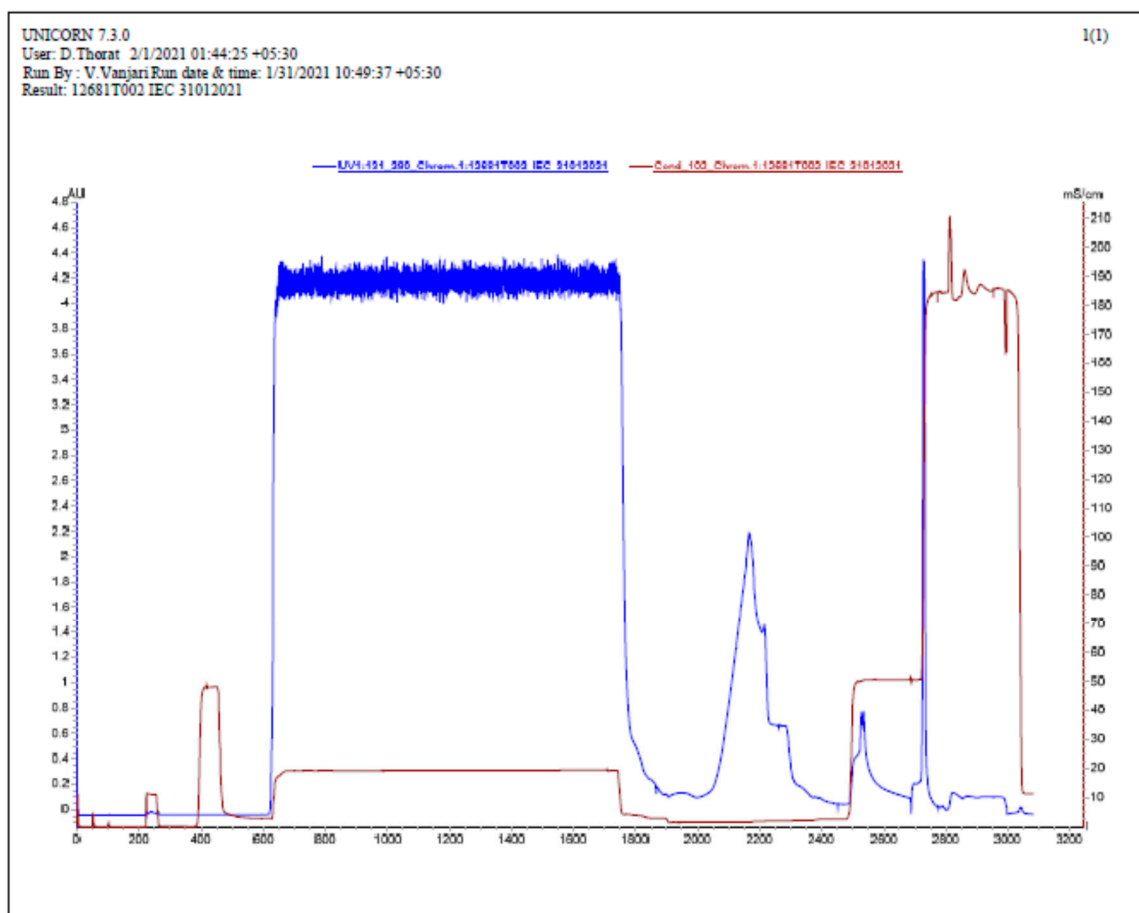
| Glycoform Mass | Glycan Structure  | Glycan Type    | Peptide Mass [M] | Peptide Sequence | Theoretical Glycopeptide Mass | $\Delta$ mass (Dalton) |
|----------------|---|----------------|------------------|------------------|-------------------------------|------------------------|
| 527.185        | (Hex) <sub>2</sub> (HexNAc) <sub>1</sub>  | -              | 1737.851         | ITNLCPFGGEVFNATR | 2266.043                      | -0.001                 |
| 656.228        | (Hex) <sub>1</sub> (HexNAc) <sub>1</sub> (NeuAc) <sub>1</sub>   | -              | 1680.829         | ITNLCPFGGEVFNATR | 2338.065                      | 0                      |
| 846.312        | (Hex) <sub>1</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>1</sub> (Pent) <sub>1</sub>   | -              | 1680.829         | ITNLCPFGGEVFNATR | 2528.149                      | 0                      |
| 876.322        | (Hex) <sub>2</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>1</sub>   | -              | 1737.851         | ITNLCPFGGEVFNATR | 2615.18                       | 0.002                  |
| 892.317        | (Hex) <sub>3</sub> (HexNAc) <sub>2</sub>  | -              | 1680.829         | ITNLCPFGGEVFNATR | 2574.154                      | 0                      |
| 933.344        | (Hex) <sub>2</sub> (HexNAc) <sub>3</sub>  | -              | 1680.829         | ITNLCPFGGEVFNATR | 2615.181                      | 0.001                  |
| 1008.365       | (Hex) <sub>2</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>1</sub> (Pent) <sub>1</sub>   | -              | 1737.851         | ITNLCPFGGEVFNATR | 2747.223                      | 0.003                  |
| 1170.417       | (Hex) <sub>3</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>1</sub> (Pent) <sub>1</sub>   | -              | 1680.829         | ITNLCPFGGEVFNATR | 2852.254                      | 0.004                  |
| 1332.47        | (Hex) <sub>4</sub> (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>1</sub> (Pent) <sub>1</sub>   | -              | 1680.829         | ITNLCPFGGEVFNATR | 3014.307                      | -0.001                 |
| 1548.545       | (Hex) <sub>1</sub> (HexNAc) <sub>1</sub> (NeuAc) <sub>1</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>                            | hybrid/complex | 1680.829         | ITNLCPFGGEVFNATR | 3230.382                      | -0.001                 |
| 1622.582       | (Hex) <sub>2</sub> (HexNAc) <sub>2</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>   | hybrid/complex | 1737.851         | ITNLCPFGGEVFNATR | 3361.44                       | -0.002                 |
| 1661.555       | (Hex) <sub>3</sub> (HexNAc) <sub>1</sub>  | -              | 1680.829         | ITNLCPFGGEVFNATR | 3343.392                      | -0.002                 |
| 1854.676       | (HexNAc) <sub>2</sub> (Deoxyhexose) <sub>2</sub> (Pent) <sub>2</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>                     | hybrid/complex | 1680.829         | ITNLCPFGGEVFNATR | 3536.513                      | -0.002                 |
| 2026.687       | (Hex) <sub>7</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>   | high_man       | 1737.851         | ITNLCPFGGEVFNATR | 3765.545                      | -0.002                 |
| 2110.794       | (HexNAc) <sub>6</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>  | hybrid/complex | 1737.851         | ITNLCPFGGEVFNATR | 3849.652                      | -0.003                 |
| 2142.809       | (HexNAc) <sub>4</sub> (Deoxyhexose) <sub>3</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>   | hybrid/complex | 1737.851         | ITNLCPFGGEVFNATR | 3881.667                      | 0.002                  |
| 2188.74        | (Hex) <sub>8</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>   | high_man       | 1737.851         | ITNLCPFGGEVFNATR | 3927.598                      | 0.004                  |
| 2262.814       | (Hex) <sub>2</sub> (HexNAc) <sub>3</sub> (Deoxyhexose) <sub>1</sub> (NeuAc) <sub>1</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub> | hybrid/complex | 1737.851         | ITNLCPFGGEVFNATR | 4001.672                      | 0                      |
| 2319.836       | (Hex) <sub>2</sub> (HexNAc) <sub>4</sub> (NeuAc) <sub>1</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>                            | hybrid/complex | 1680.829         | ITNLCPFGGEVFNATR | 4001.673                      | 0                      |
| 2459.931       | (HexNAc) <sub>7</sub> (Deoxyhexose) <sub>1</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>   | hybrid/complex | 1737.851         | ITNLCPFGGEVFNATR | 4198.789                      | 0                      |
| 2516.952       | (HexNAc) <sub>8</sub> + (Man) <sub>3</sub> (GlcNAc) <sub>2</sub>  | hybrid/complex | 1680.829         | ITNLCPFGGEVFNATR | 4198.789                      | 0                      |

**Supplementary Table S9: Batch release summary**

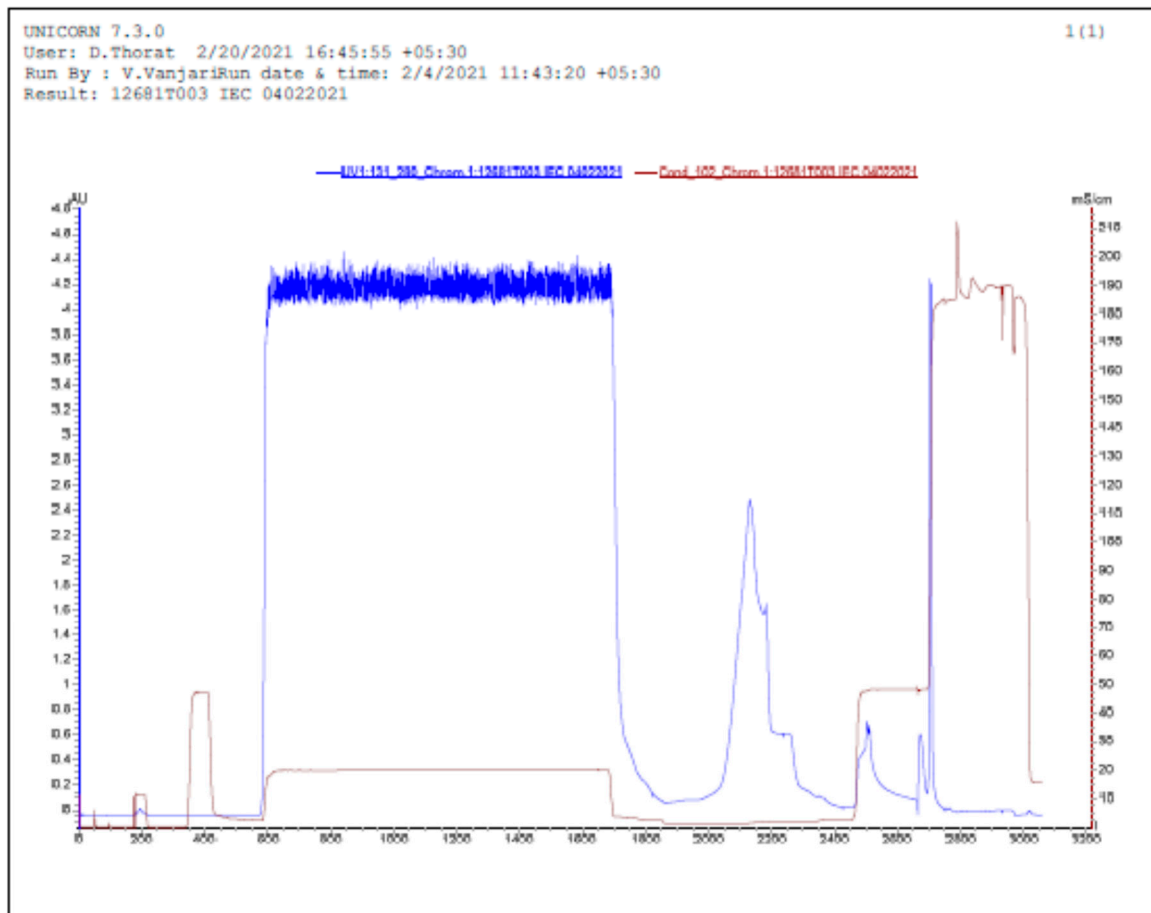
| Test Name                                 | Specification/<br>Acceptance<br>Criteria   | Unit          | Batch No.   |   |   |  |
|---|--|---------------|---|---|---|--|
|   |  |               | 12681T002   | 12681T003   | 12681T004   | 12681T005  |
| Appearance                                | Clear Colourless to yellowish solution free from visible particles   | NA            | Clear Colourless solution free from visible particles             | Clear Colourless solution free from visible particles             | Clear Colourless solution free from visible particles             | Clear Colourless solution free from visible particles            |
| pH  | 7.00 -8.00   | NA            | 7.17  | 7.26  | 7.2   | 7.22   |
| Identity By ELISA                         | Positive Reaction for RBD Spytag with anti-RBD Antibody In ELISA   | NA            | Positive reaction for RBD Spytag with anti-RBD Antibody In ELISA  | Positive reaction for RBD Spytag with anti-RBD Antibody In ELISA  | Positive reaction for RBD Spytag with anti-RBD Antibody In ELISA  | Positive reaction for RBD Spytag with anti-RBD Antibody In ELISA |
| Purity By SDS -PAGE                       | Not less than 90%  | %             | 99.81   | 100   | 100   | 100  |
| Purity By HPLC                            | Not Less than 90 %   | %             | 100   | 100   | 100   | 100  |
| Identity By SDS PAGE and Western Blotting | Molecular weight of main band should not be less than 24 kDa and band should be identified by western Blot | NA            | Molecular weight of main band is 32.18 kDa and band is identified | Molecular weight of main band is 27.07 kDa and band is identified | Molecular weight of main band is 27.31 kDa and band is identified | Molecular weight of main is 27.67 kDa and band is identified     |
| Protein Content                           | Not less than 10 mg/mL   | mg/mL         | 13.48   | 16.78   | 15.01   | 13.29  |
| Endotoxin Content                         | Less than or equal to 4 EU/MCG Protein   | EU/μG Protein | <0.05   | <0.05   | <0.05   | <0.05  |
| Antigenic Ratio                           | Not less than 0.700  | NA            | 0.928   | 0.973   | 1.016   | 1.341  |
| Antigenic Content                         | Not less than 7 mg/mL  | mg/mL         | 12.51   | 16.33   | 15.25   | 17.82  |
| Bioburden                                 | Less than or equal to 10CFU/10ml   | CFU/10mL      | 0   | 0   | 0   | 0  |
| Host Cell Protein (HCP) content           | Less than or equal to 5% of Protein  | %             | 0.01  | 0.01  | 0.01  | 0.01   |
| Host cell DNA (HCD) Content               | Less than or equal to 1Ng/MCG of Protein   | ng/ μg        | 0.1   | 0.08  | 0.09  | 0.07   |



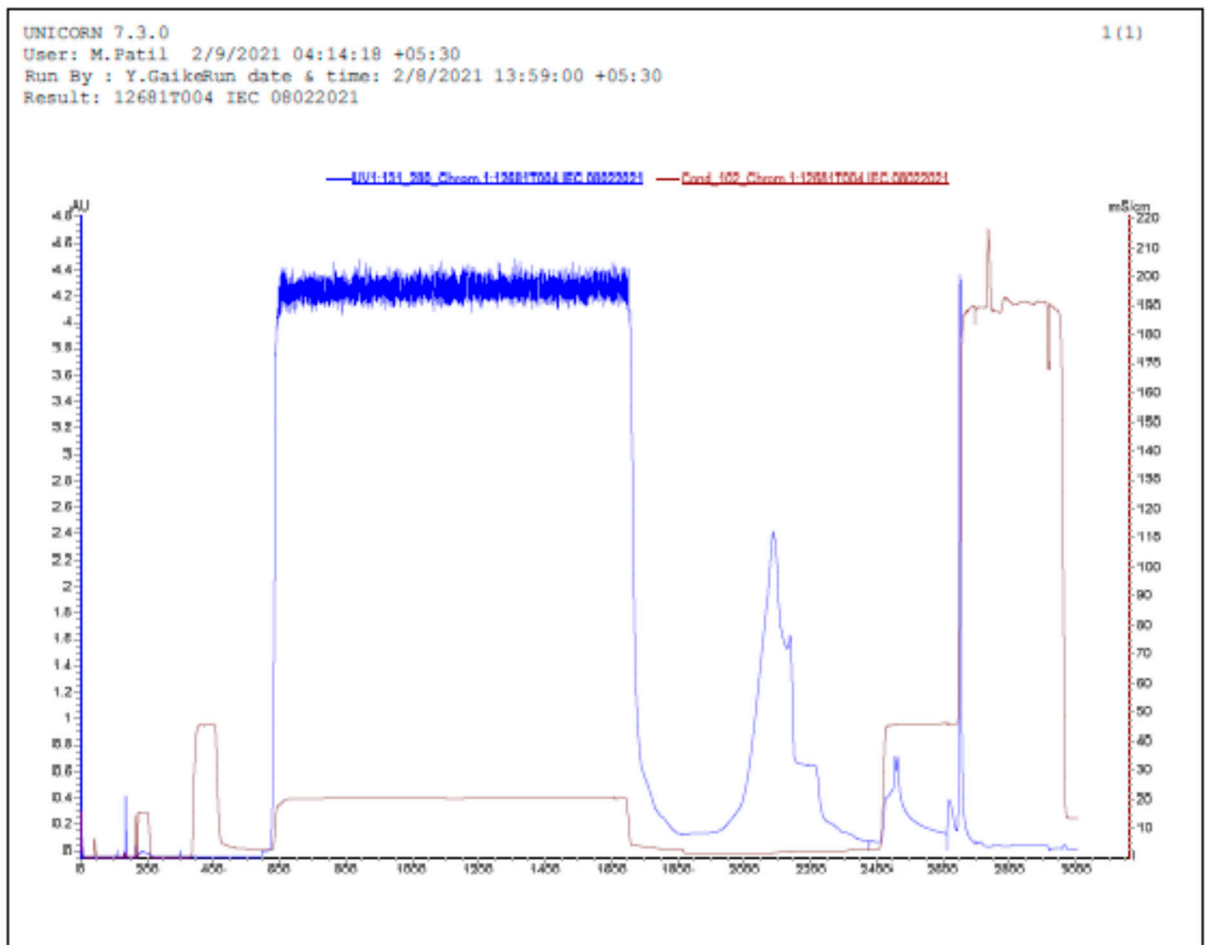
**Supplementary Figure S1. Mixed-mode chromatography (B. No. 12680T002)**



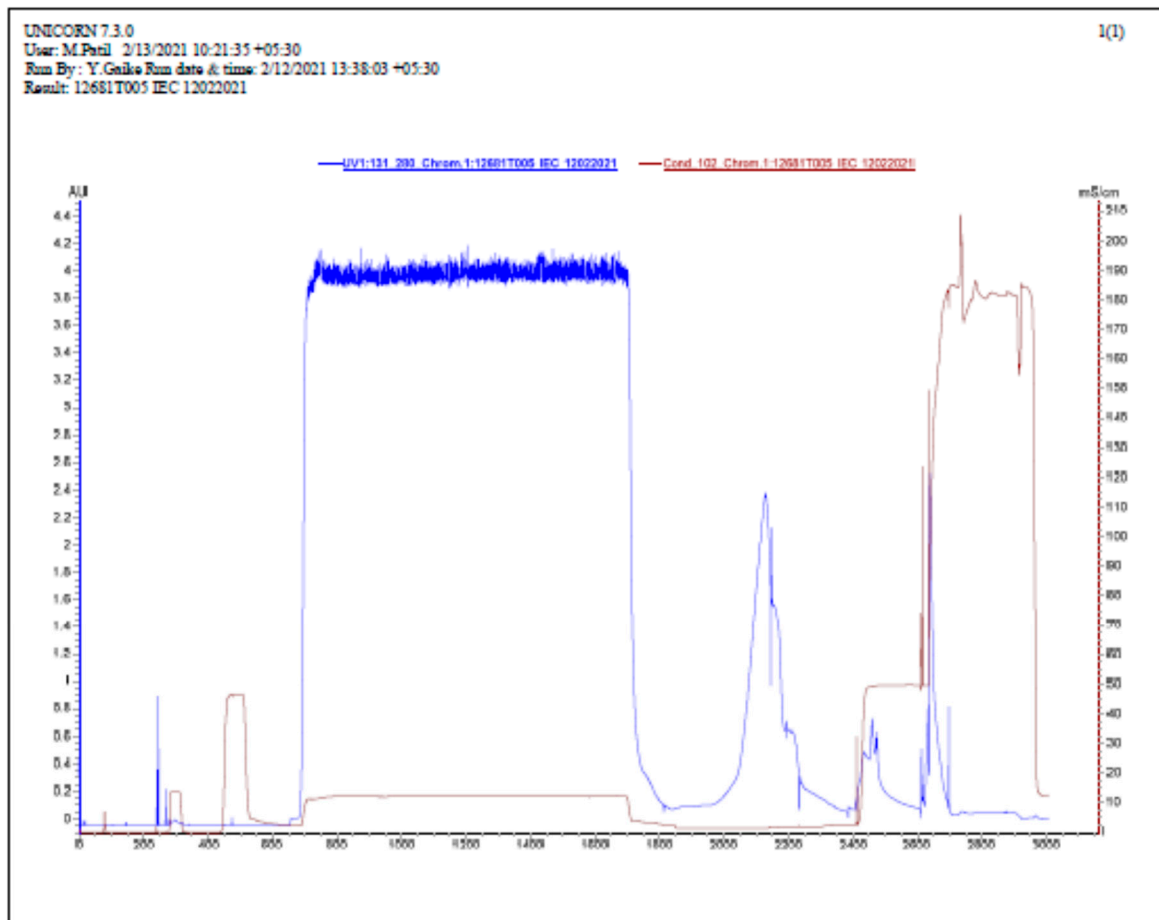
**Supplementary Figure S2. Mixed-mode chromatography (B. No. 12680T003)**



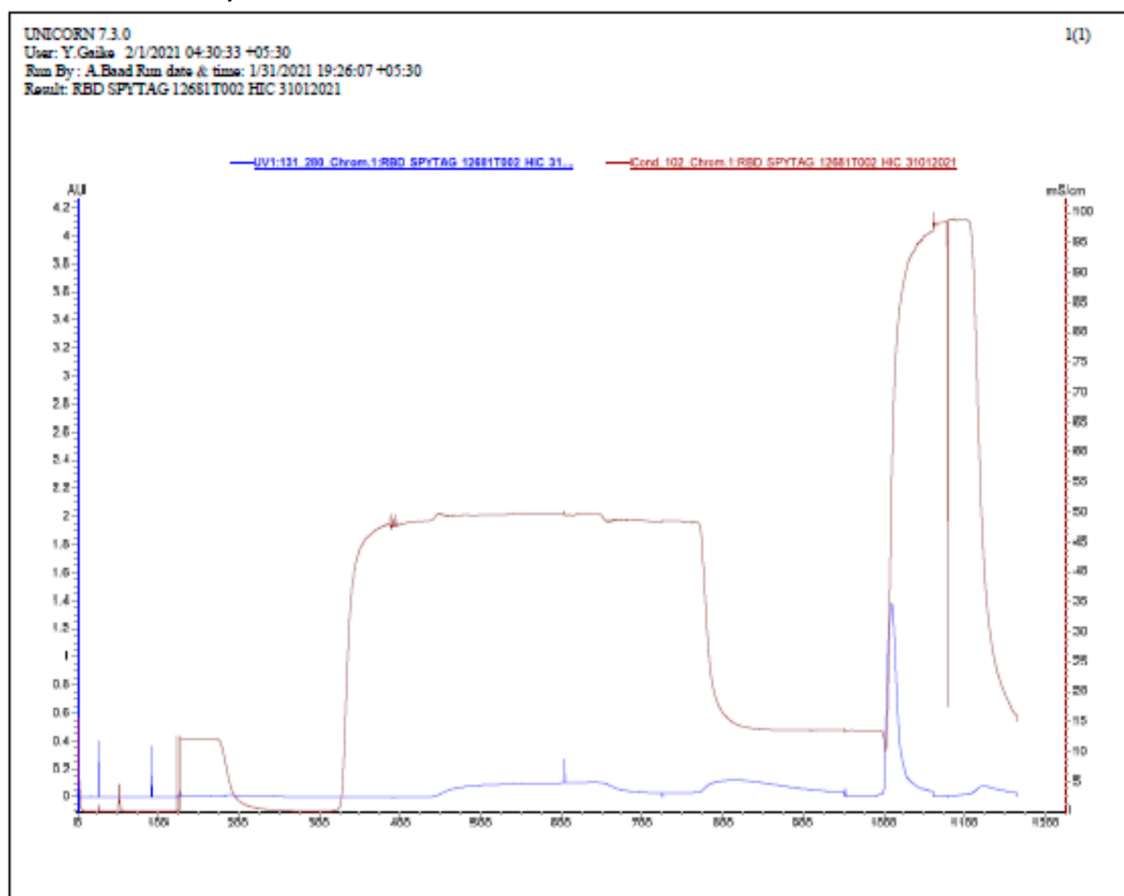
**Supplementary Figure S3. Mixed-mode chromatography (B. No. 12680T004)**



**Supplementary Figure S4. Mixed-mode chromatography (B. No. 12680T005)**

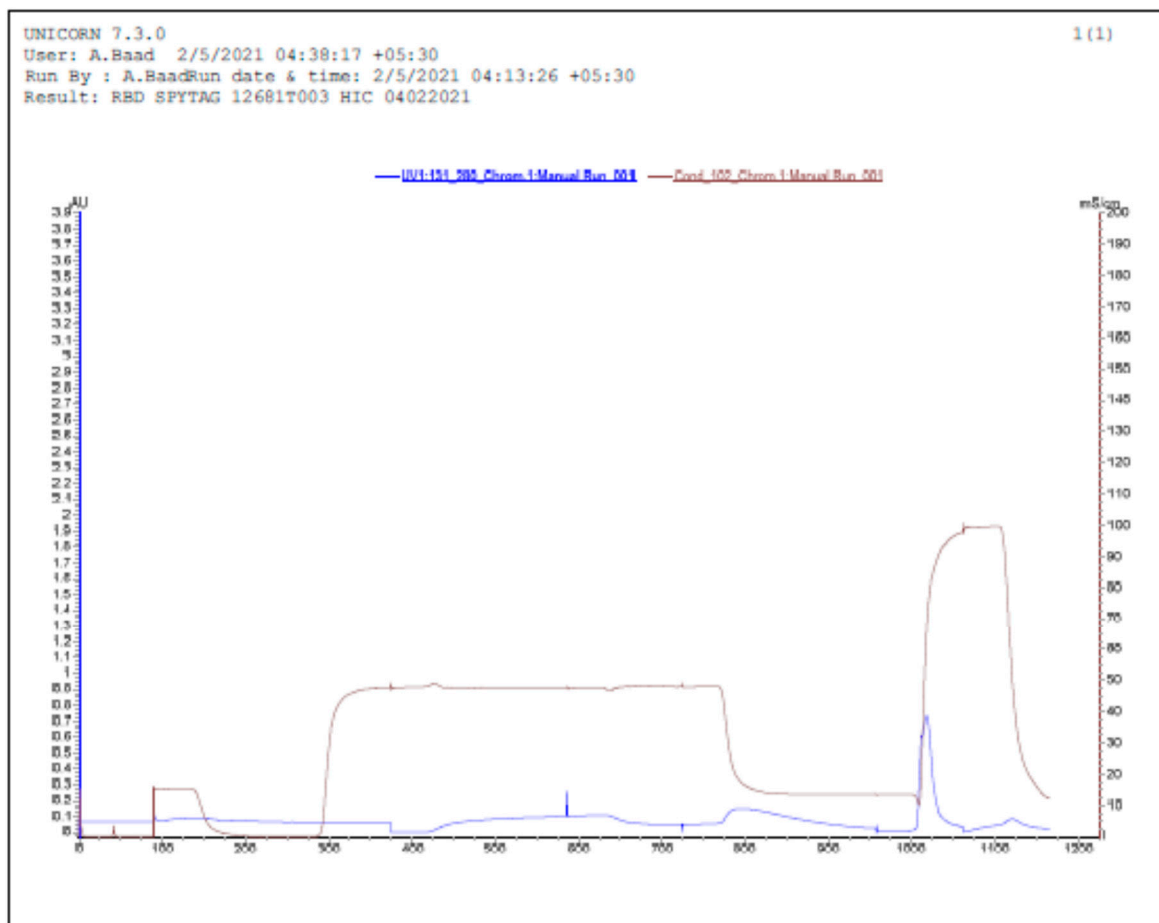


**Supplementary Figure S5. Hydrophobic Interaction chromatography (B. No. 12680T002)**

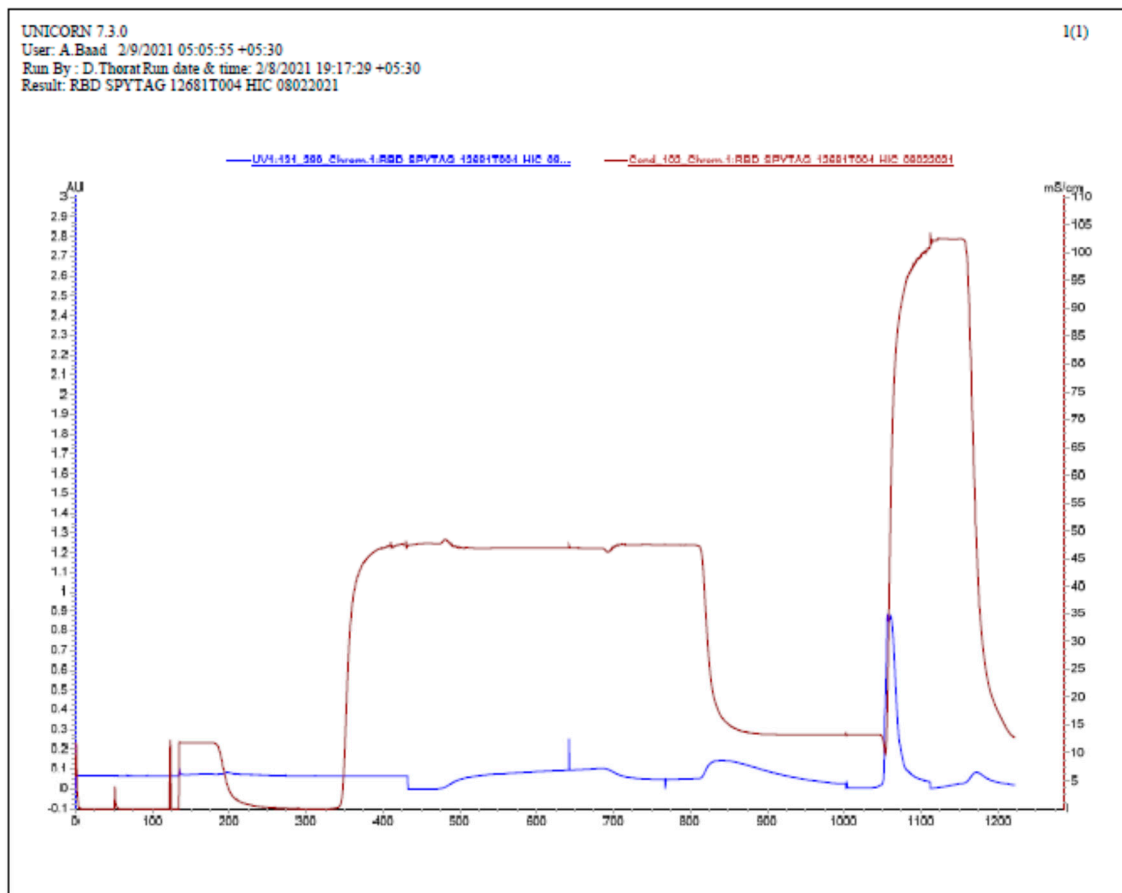




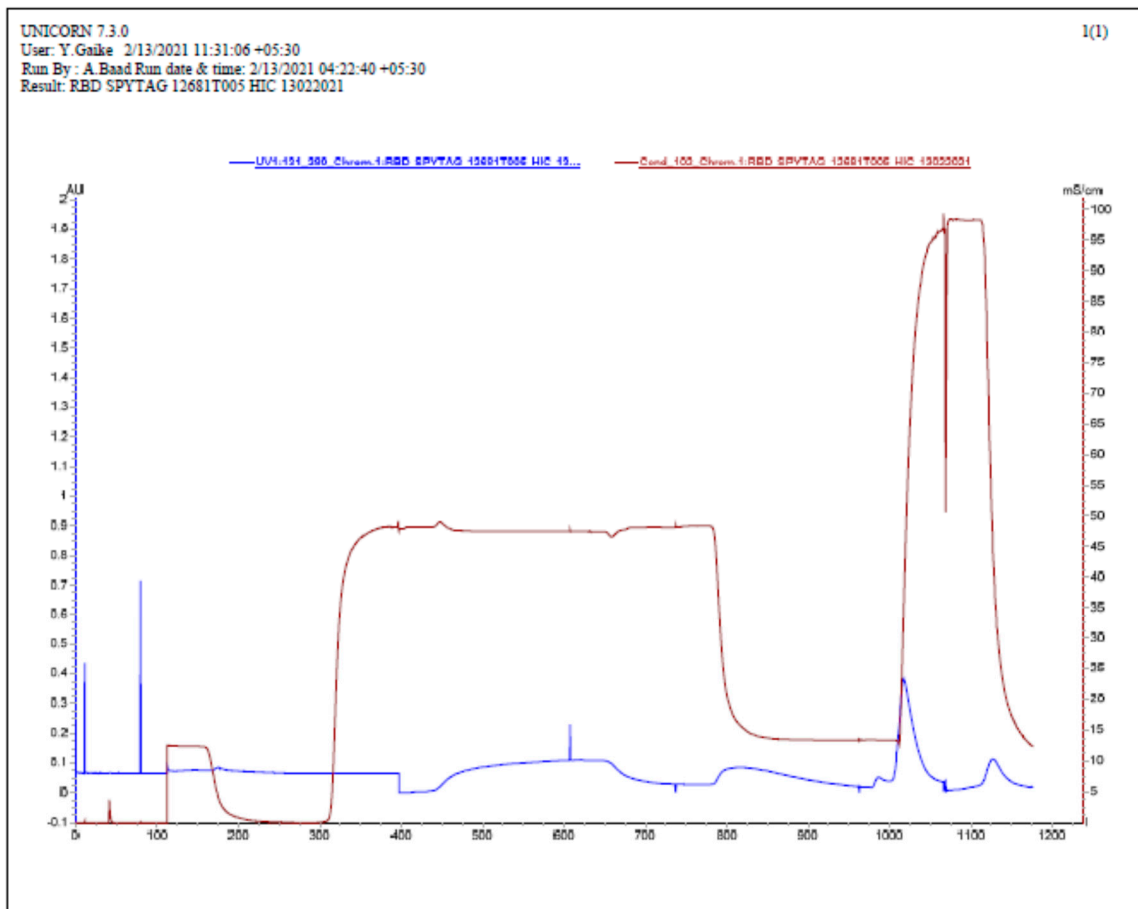
**Supplementary Figure S6. Hydrophobic Interaction chromatography (B. No. 12680T003)**



# Supplementary Figure S7. Hydrophobic Interaction chromatography (B. No. 12680T004)



**Figure S8. Hydrophobic Interaction chromatography (B. No. 12680T005)**



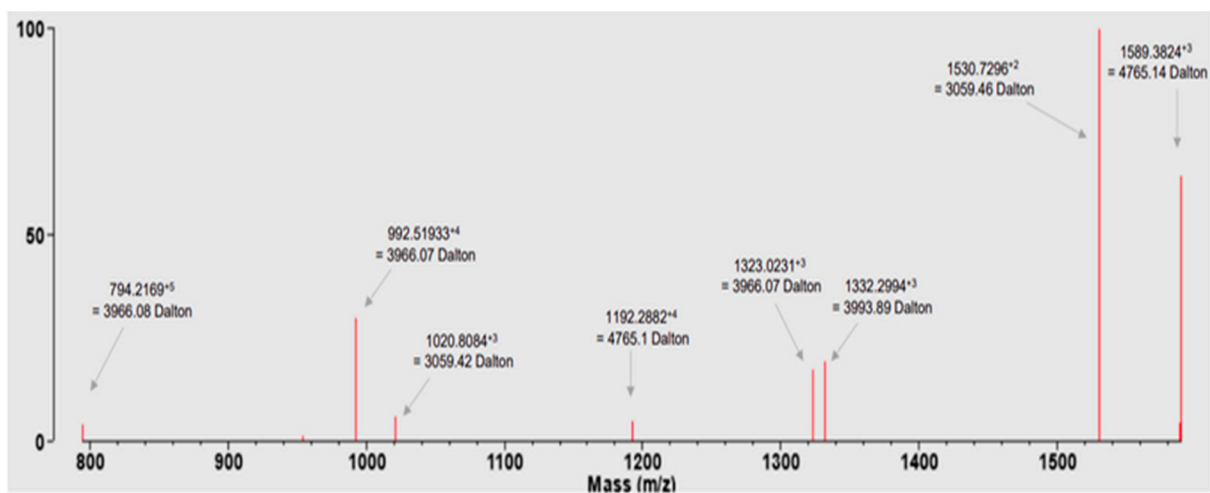
Supplementary Figure S9: Theoretical Construct of Di-Sulfide linked peptides that can be obtained upon Trypsin digestion of the protein at C terminal Arginine and lysine



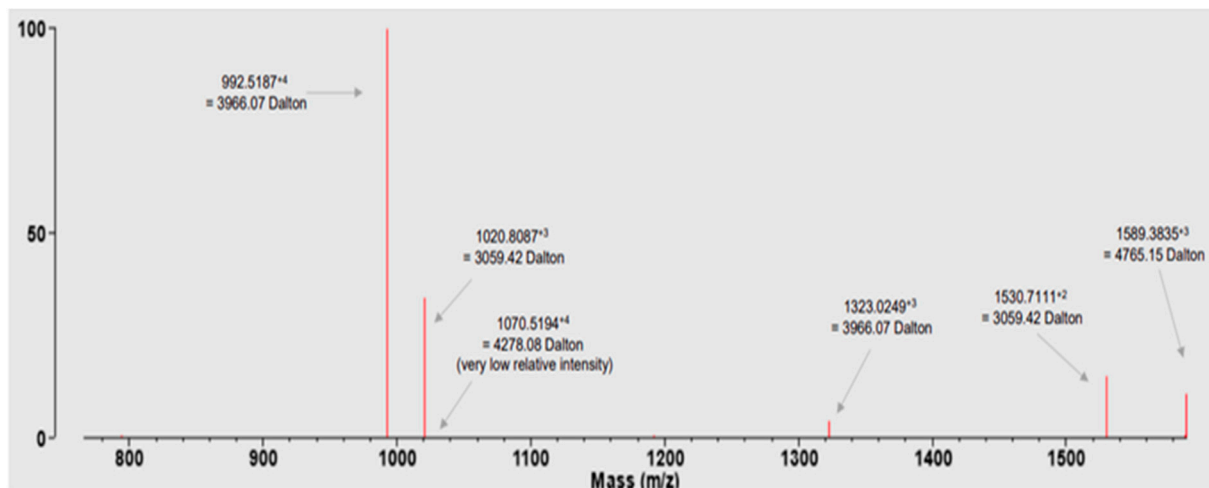
Arrangement of disulfide linkage in RBD protein



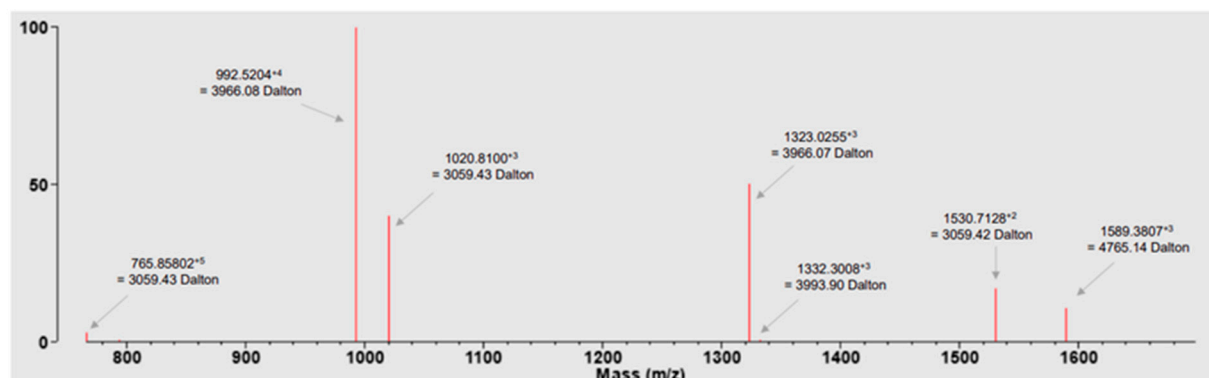
Supplementary Figure S10 m/z spectrum of Di-Sulfide linked peptides obtained from B. No. 12681T002



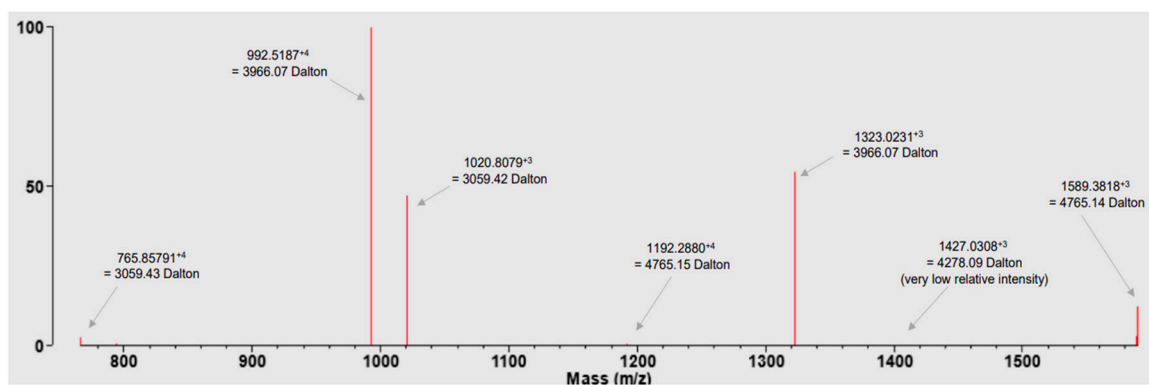
Supplementary Figure S11 m/z spectrum of Di-Sulfide linked peptides obtained from B. No. 12681T003



Supplementary Figure S12 m/z spectrum of Di-Sulfide linked peptides obtained from B. No. 12681T004

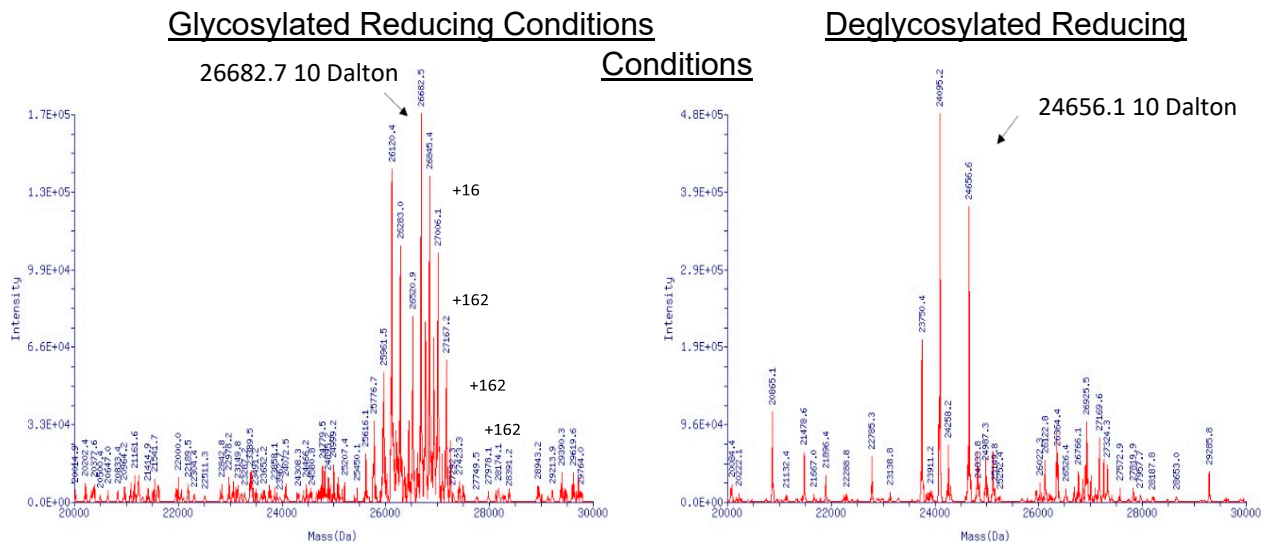


Supplementary Figure S13 m/z spectrum of Di-Sulfide linked peptides obtained from B. No. 12681T005

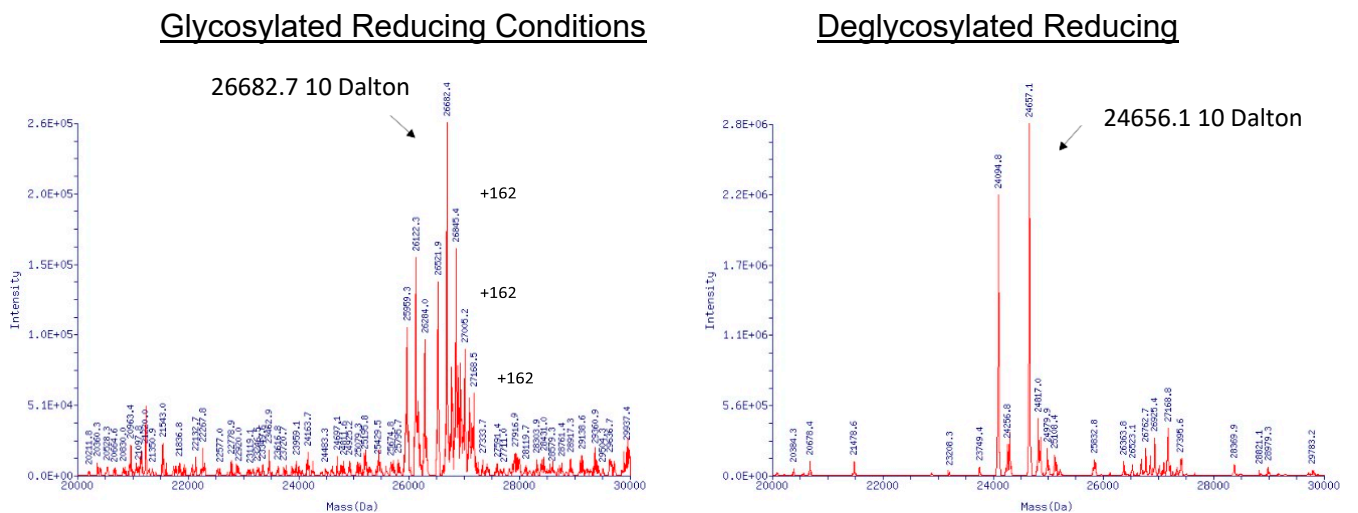




**Figure S14. Deconvoluted Molecular Mass of 'RBD SARS-CoV2 Ag with Spytag' (B. No. 12680T002)**



**Figure S15. Deconvoluted Molecular Mass of 'RBD SARS-CoV2 Ag with Spytag' (B. No. 12680T003)**



## Figure S16: Sequence details of RBD construct:

(**Reference:** Neil, C. Dalvie.; et al. Scalable, Methanol-Free Manufacturing of the SARS-CoV-2 Receptor-Binding Domain in Engineered *Komagataella Phaffii*. Biotechnology and Bioengineering 2022, 119,2,657–62)

Amino Acid Sequence:

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYS DLEGDFDVAVL PFSNS  
 TNNGLLFINTT IASIAAKEEGVSLEKRITNLCPFGEVFNATRFASVYAWN RKRISNCV  
 ADYSVL YNSASFSTFKCYGV SPTKLN DLCTNVYADSFVIRGDEV RQIAPGQTG KIA  
 DYN YKL PDDFTGCV IAWNSN NLD SKVGGN YNYLYRLFRKSN LKPFERDISTE IYQA  
 GSTPCNGVEGFNCYFPLQSYGFQPTNGVG YQPYRVV VLSFELLHAPATVCGPKKS  
 TGGDGGDGGDGGAHIVMVDAYKPTK

|                |             |        |         |
|----------------|-------------|--------|---------|
| Signal Peptide | RBD protein | Linker | Spy tag |
|----------------|-------------|--------|---------|

## Figure S17. Expression Construct scheme and strain information:

Details: L1 to L2:pAOX1 promoter.

L2 to L3: RBD\_linker\_ spy tag

L3 to L4: AOX1\_TT

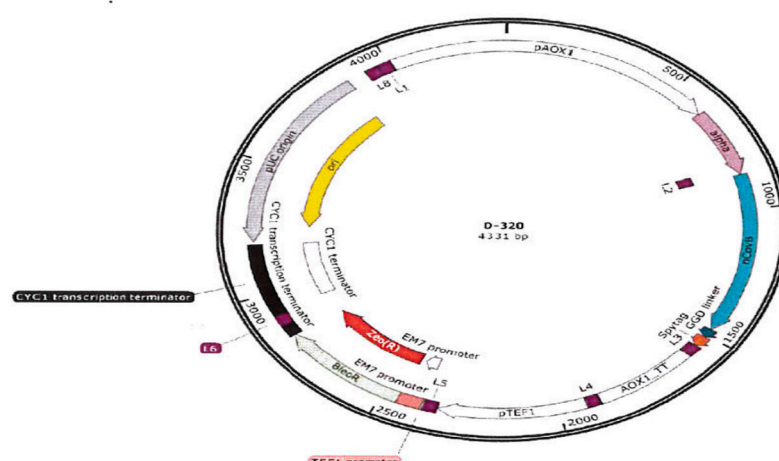
L4 to L5: pTEF Promoter

L5 to L6:Em7 promoter, Zeocin Resistance

L6: CYC1 terminator

L-7 to L-8: pUC origin of replication

L-8 to L1:pAOX1 promoter.



## Strain Information:

Strain S-380 is derived from base strain NRRL Y-11430 with genomic modification, which has been reported before.

Strain Information RNA sequencing data is available in the NCBI Gene Expression Omnibus, accession GSE183408  
(**GSE183408\_phaffi\_transcripts\_plusTransgenes011021.fa.gz**)