

Table S1: List of synthesized peptides covering the entire sequence of DTx. (F3,F4,F5, F11,F12,F13 positive controls and F9,F17 negative controls.

| Spot | Sequence | Spot | Sequence | Spot | Sequence |
|------|-------------------|------|------------------|------|------------------|
| A1 | GS GSGMSRKL FASIL | B1 | VLALKVDNAETIKKE | C1 | DKTKTKIESLKEHGP |
| A2 | MSRKL FASILIGALL | B2 | VDNAETIKKELGLSL | C2 | KIESLKEHGPIKNKM |
| A3 | FASILIGALLGIGAP | B3 | TIKKELGLSLTEPLM | C3 | KEHGPIKNKMSESPN |
| A4 | IGALLGIGAPPSAHA | B4 | LGLSLTEPLMEQVGT | C4 | IKNKMSESPNKTVSE |
| A5 | GIGAPPSAHAGADDV | B5 | TEPLMEQVGTEEFIK | C5 | SESPNKTVSEEKAKQ |
| A6 | PSAHAGADDVVDSSK | B6 | EQVGTEEFIKRFGDG | C6 | KTVSEEKAKQYLEEF |
| A7 | GADDVVDSSKSFVME | B7 | EEFIKRFGDGASRVV | C7 | EKAKQYLEEFHQ TAL |
| A8 | VDSSKSFVMENFSSY | B8 | RFGDGASRVVLSLPF | C8 | YLEEFHQTALEHPEL |
| A9 | SFVMENFSSYHG TKP | B9 | ASRVVLSLPFAEGSS | C9 | HQTALEHPELSELKT |
| A10 | NFSSYHG TKPGYVDS | B10 | LSLPFAEGSSSVEYI | C10 | EHPELSELKTVTGTN |
| A11 | HG TKPGYVDSIQKGI | B11 | AEGSSSVEYINNWEQ | C11 | SELKTVTGTNPVFAG |
| A12 | GYVDSIQKGIQPKS | B12 | SVEYINNWEQAKALS | C12 | VTGTNPVFAGANYAA |
| A13 | IQKGIQPKSGTQGN | B13 | NNWEQAKALSVELEI | C13 | PVFAGANYAAWAVNV |
| A14 | QKPKSGTQGN YDDDW | B14 | AKALSVELEINFETR | C14 | ANYAAWAVNVAQVID |
| A15 | GTQGN YDDDWKGFYS | B15 | VELEINFETRGRGQ | C15 | WAVNVAQVIDSETAD |
| A16 | YDDDWKGFYSTDNKY | B16 | NFETRGRGQDAME | C16 | AQVIDSETADNLEKT |
| A17 | KGFYSTDNKYDAAGY | B17 | GKRGQDAMEYMAQA | C17 | SETADNLEKTTAALS |
| A18 | TDN KYDAAGYSVDNE | B18 | DAMEYMAQACAGNR | C18 | NLEKTTAALSILPGI |
| A19 | DAAGYSVDNENPLSG | B19 | YMAQACAGNRVRRSV | C19 | TAALSILPGIGSVMG |
| A20 | SVDNENPLSGKAGGV | B20 | CAGNRVRRSVGSSLS | C20 | ILPGIGSVMGIADGA |
| A21 | NPLSGKAGGVVKVTY | B21 | VRRSVGSSLSCLND | C21 | GSMGIADGAVHHNT |
| A22 | KAGGVVKVTYPGLTK | B22 | GSSLSCLNDWDVIR | C22 | IADGAVHHNTEEIVA |
| A23 | VKVTYPGLTKVLALK | B23 | CINLDWDVIRDKTKT | C23 | VHHNTEEIVAQSIAL |
| A24 | PGLTKVLALKVDNAE | B24 | WDVIRDKTKTKIESL | C24 | EEIVAQSIALSSLMV |
| | | | | | |
| D1 | QSIALSSLMVAQAIP | E1 | SVNGRKIRMRCRAID | F1 | |
| D2 | SSLMVAQAIPLVGEL | E2 | KIRMRCRAIDGDVTF | F2 | |
| D3 | AQAIPLVGELVDIGF | E3 | CRAIDGDVTFCRPKS | F3 | KEVPALTAVETGATN |
| D4 | LVGELVDIGFAAYNF | E4 | GDVTFCRPKSPVYVG | F4 | |
| D5 | VDIGFAAYNFVESII | E5 | CRPKSPVYVGNGVHA | F5 | GYPKDGNAFNLDRI |
| D6 | AAYNFVESIINLFQV | E6 | PVYVGNGVHANLHVA | F6 | |
| D7 | VESIINLFQVVHNSY | E7 | NGVHANLHVAFHRSS | F7 | YDYDVPDYAGYPYDV |
| D8 | NLFQVVHNSYNRPAY | E8 | NLHVAFHRSSSEKIH | F8 | |
| D9 | VHNSYNRPAYSPGHK | E9 | FHRSSSEKIHSNEIS | F9 | QEV RKYFCV |
| D10 | NRPAYSPGHKTQPFL | E10 | SEKIHSNEISSDSIG | F10 | |
| D11 | SPGHKTQPFLHDGYA | E11 | SNEISSDSIGVLGYQ | F11 | KEVPALTAVETGATN |
| D12 | TQPFLHDGYAVSWNT | E12 | SDSIGVLGYQKTVDH | F12 | |
| D13 | HDGYAVSWNTVEDSI | E13 | VLGYQKTVDH TKVNS | F13 | GYPKDGNAFNLDRI |
| D14 | VSWNTVEDSIIRTGF | E14 | KTVDH TKVNSKLSLF | F14 | |
| D15 | VEDSIIRTGFQGESG | E15 | TKVNSKLSLFFEIKS | F15 | YDYDVPDYAGYPYDV |
| D16 | IRTGFQGESGHDIKI | E16 | KLSLFFEIKSGSGSG | F16 | |
| D17 | QGESGHDIKITAENT | E17 | | F17 | QEV RKYFCV |
| D18 | HDIKITAENTPLPIA | E18 | | F18 | |
| D19 | TAENTPLPIAGVLLP | E19 | | F19 | |
| D20 | PLPIAGVLLPTIPGK | E20 | | F20 | |
| D21 | GVLLPTIPGKLDV NK | E21 | | F21 | |
| D22 | TIPGKLDV NKSKTHI | E22 | | F22 | |
| D23 | LDV NKSKTHISVNGR | E23 | | F23 | |
| D24 | SKTHISVNGRKIRMR | E24 | | F24 | |

Figure 1. List of the synthesized peptides of DTX covering the sequence aa518 to aa547, and probed with miVS (A), huVS (B), and hoThe sera (C). In black, the sequences considered reactive to antibodies are marked, using a 50% cut-off. The membrane is shown in figure 2.

| A | | B | | C | |
|-----|-----------------|-----|-----------------|-----|-----------------|
| A1 | RSSSEKIHSNEISSD | A1 | RSSSEKIHSNEISSD | A1 | RSSSEKIHSNEISSD |
| A2 | SSSEKIHSNEISSDS | A2 | SSSEKIHSNEISSDS | A2 | SSSEKIHSNEISSDS |
| A3 | SSEKIHSNEISSDSI | A3 | SSEKIHSNEISSDSI | A3 | SSEKIHSNEISSDSI |
| A4 | SEKIHSNEISSDSIG | A4 | SEKIHSNEISSDSIG | A4 | SEKIHSNEISSDSIG |
| A5 | EKIHSNEISSDSIGV | A5 | EKIHSNEISSDSIGV | A5 | EKIHSNEISSDSIGV |
| A6 | KIHSNEISSDSIGVL | A6 | KIHSNEISSDSIGVL | A6 | KIHSNEISSDSIGVL |
| A7 | IHSNEISSDSIGVLG | A7 | IHSNEISSDSIGVLG | A7 | IHSNEISSDSIGVLG |
| A8 | HSNEISSDSIGVLGY | A8 | HSNEISSDSIGVLGY | A8 | HSNEISSDSIGVLGY |
| A9 | SNEISSDSIGVLGYQ | A9 | SNEISSDSIGVLGYQ | A9 | SNEISSDSIGVLGYQ |
| A10 | NEISSDSIGVLGYQK | A10 | NEISSDSIGVLGYQK | A10 | NEISSDSIGVLGYQK |
| A11 | EISSDSIGVLGYQKT | A11 | EISSDSIGVLGYQKT | A11 | EISSDSIGVLGYQKT |
| A12 | ISSDSIGVLGYQKTV | A12 | ISSDSIGVLGYQKTV | A12 | ISSDSIGVLGYQKTV |
| A13 | SSDSIGVLGYQKTV | A13 | SSDSIGVLGYQKTV | A13 | SSDSIGVLGYQKTV |
| A14 | SDSIGVLGYQKTVDH | A14 | SDSIGVLGYQKTVDH | A14 | SDSIGVLGYQKTVDH |
| A15 | DSIGVLGYQKTVDHT | A15 | DSIGVLGYQKTVDHT | A15 | DSIGVLGYQKTVDHT |

Figure S2: Alignment of the primary structure of DTX [(1-*C. diphtheria* (Q6NK15), 2-*C. ulcerans* (AQA1Y0 HBB0) and 3-*C. pseudotuberculosis* (WP_014654963.1)] showing in grey the epitopes (CB/DTx1-20) identified by Spot-synthesis.

| | | | | | | | |
|--|------------|------------|-------------|------------|------------|-------------|------------|
| 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 |
| 1-MSRKL FASIL | IGALLGIGAP | PSAHAGADDV | VDSSKSFVME | NFSSYHGTGP | GYVDSIQKGI | QKPKSGTQGN | YDDDWKGFYS |
| 2-MNRKL FASIL | IGALLGIGTP | LSAHASADDV | VDSSKSFVME | NFSSYHGTGP | GYVDSIQKGI | QKPKSGTQGN | YDDDWKGFYS |
| 3-MNRKL FALIL | IGALLGIGAP | LSAHASVDDV | VDSSKSFVME | NFSSYHGTGP | GYVDSIQKGI | QKPKSGTQGN | YDDDWKGFYS |
| -----CB/Tx-1-----CB/Tx-2-----CB/Tx-3-----CB/Tx-4 | | | | | | | |
| 90 | 100 | 110 | 120 | 130 | 140 | 150 | 160 |
| 1-TDNKYDAAGY | SVDNENPLSG | KAGGVVKVTY | PGLTKVLALK | VDNAETIKKE | LGLSLTEPLM | EQVGTEEFIK | RFGDGASRVV |
| 2-TDNKYDAAGY | SVDNENPLSG | KAGGVVKVTY | PGLTKILALK | VDNAETIKKE | LGLSLTEPLM | EQVGSEEFIK | RFGDGASRVV |
| 3-TDNKYDAAGY | SVDNENPLSG | KAGGVVKVTY | PGLTKILALK | VDNAETIKKE | LGLSLTEPLM | EQVGSEEFIK | RFGDGASRVV |
| -----CB/Tx-5-----CB/Tx-6-----CB/Tx-7 | | | | | | | |
| 170 | 180 | 190 | 200 | 210 | 220 | 230 | 240 |
| 1-LSLPFAEGSS | SVEYINNWEQ | AKALSVELEI | NFETRGRKRG | DAMYEYMAQA | CAGNRVRRSV | GSSLSCINLD | WDVIRDKTKT |
| 2-LSLPFAEGSS | SVEYINNWEQ | AKALSVELEI | NFETRGRKRG | DAMYEYMAQS | CAGNRVRRSV | GNSSSCINLD | WDAIRDKTKT |
| 3-LSLPFAEGSS | SVEYINNWEQ | AKALSVELEI | NFENRGRKRG | DAMYEYMAQS | CAGNRIRRSV | SNSSSCINLD | WDAIRDKTKA |
| -----CB/Tx-8-----CB/Tx-9-----CB/Tx-10-----CB/Tx-11 | | | | | | | |
| 250 | 260 | 270 | 280 | 290 | 300 | 310 | 320 |
| 1-KIESLKEHGP | IKNKMSSEPN | KTVSEEKAKQ | YLEEFHQ TAL | EHPSELSELT | VTGTNPVFAG | ANYAAWAVNV | AQVIDSETAD |
| 2-KIESLKEHGP | IKNKMSSEPN | KAVSEEKAKQ | YLEEFHQ TAL | EHPSELSELT | VTGTNSVFAG | ANYAAWAVNV | AQVIDSETAD |
| 3-KIESLKENG P | IKNKMSSEPN | KTVSEEKAKQ | YLEEFHQ TAL | EHPSELSELT | VAETNSVFAG | ANYAAWAVNV | AQVIDSKTAD |
| -----CB/Tx-10-----CB/Tx-11-----CB/Tx-12-----CB/Tx-13 | | | | | | | |
| 330 | 340 | 350 | 360 | 370 | 380 | 390 | 400 |
| 1-NLEKTTAALS | ILPGIGSVMG | IADGAVHHNI | EEIVAQSIAL | SSLMVAQAIP | LVGELVDIGF | AAYNFVESII | NLFQVVHNSY |
| 2-NLEKTTAALS | ILPGIGSVMG | IADGAVHHNT | EEIVAQSIAL | SSLMVAQAIP | LVGELVDIGF | AAYNFVESII | NLFQVVHNSY |
| 3-NLEKTTAALS | ILPGIGSVMG | IADGAVHHNT | EEIVAQSIAL | SSLMIAQAIP | LVGELVDIGF | AAYNFVESII | NLFQVIHNSY |
| -----CB/Tx-14-----CB/Tx-15-----CB/Tx-16-----CB/Tx-17 | | | | | | | |
| 410 | 420 | 430 | 440 | 450 | 460 | 470 | 480 |
| 1-NRPAYSPGHK | TQPFLHDGYA | VSWNTVEDSI | IRTGFQGESG | HDIKITAENT | PLPIAGVLLP | TIPGKLDV NK | SKTHISVNGR |
| 2-NRPAYSPGHK | TQPFLHDGYA | VSWNTVEDSI | IQTGFQGESG | HDIKITAENT | PLPIAGVLLP | TIPGKLDV NK | SKTHISVNGR |
| 3-NRPAYSPGHK | TQPFLHDGYA | VSWNTVEDSI | IKTGFQGESG | HDIKITAENT | PLPIAGVLLP | TITGKLDV NK | SKTHISVNGR |
| -----CB/Tx-16-----CB/Tx-17-----CB/Tx-18-----CB/Tx-19 | | | | | | | |
| 490 | 500 | 510 | 520 | 530 | 540 | 550 | 560 |
| 1-KIRMRCAID | GDVTFPCRPS | PVYVGNGVHA | NLHVAFHRSS | SEKIHSNEIS | SDSIGVLGYQ | KIVDHTKVNS | KLSLFFEIKS |
| 2-KIRMRCAID | GDVTFPCRPS | PVYVGNGVHA | NLHVAFHRSS | SEKIHSNEIS | SDSIGVLGYQ | KIVDHTKVNS | KLSLFFEIKS |
| 3-KIRMQCAID | GNVTFPCRPS | PVYVGKGVHA | NLHVAFHRSN | SEKIQSNEIL | SDSIGILGYQ | KIVDHTKVNS | KLSLFFEIKS |
| -----CB/Tx-19-----CB/Tx-20-----CB/Tx-21 | | | | | | | |