

Supplementary Data

Table S1. Sequences of candidate signature peptides for antigen quantification.

DTd				
	Sequence	Position in antigen	MH⁺ (monoisotopic)	Antigen intensity with 1:20 w/w Asp-N ratio
Pep_DTd_01	DSIIRTGFQGEGSH	404-417	1503.7187	1.22E8
Pep_DTd_02	DSIGVLGYQKTV	539-550	1279.6892	7.58E7
Pep_DTd_03	DAAGYSV ²	62-68	682.3042	NF
Pep_DTd_04	DSETA ¹	291-295	522.2042	6.27E5
Pep_DTd_05	DHTKVNSKLSLFFEIKS ²	520-536	1993.0753	NF
TTd				
Pep_TTd_01	DLYEKTIN	290-297	995.5044	3.64E7
Pep_TTd_02	DTEGFNIESK	396-405	1139.5215	5.81E7
Pep_TTd_03	DPNYLRT	74-80	878.4367	1.02E7
Pep_TTd_04	DTEYYLIPVASSSK	1125-1138	1572.7792	1.90E7
Pep_TTd_05	DPVKIPNLL	381-389	1008.6088	4.68E6
Pep_TTd_06	DVQLKNIT	1139-1146	930.5255	2.85E7
FHA				
Pep_FHA_01	DAGLAGPSAVAAPAVGAA	2305-2322	1465.7645	3.92E7
Pep_FHA_02	DVGLEKRL	2214-2221	929.5415	9.61E7
Pep_FHA_03	DATLTLAQGTQR	1661-1672	1274.6699	2.69E7
Pep_FHA_04	DLTAEQSLIEVGK	1855-1867	1402.7424	5.80E7
Pep_FHA_05	DLRTVYAKQA	2076-2085	1164.6372	4.43E7
PTd				
Pep_PTd_01	DGTPGGAF	309-316	721.3151	5.84E7
Pep_PTd_02	DSPYPGTPG	772-780	890.3890	5.70E7
Pep_PTd_03	DNNFYGAASSYFEYV	68-82	1746.7282	7.92E6
Pep_PTd_04	DGTYLGGEYGGVIK	295-308	1428.7005	1.98E7
Pep_PTd_05	DAGHEH ^{1,2}	740-745	665.2638	NF

¹Not antigen-specific

²peptides not suitable as target peptide based on intensity

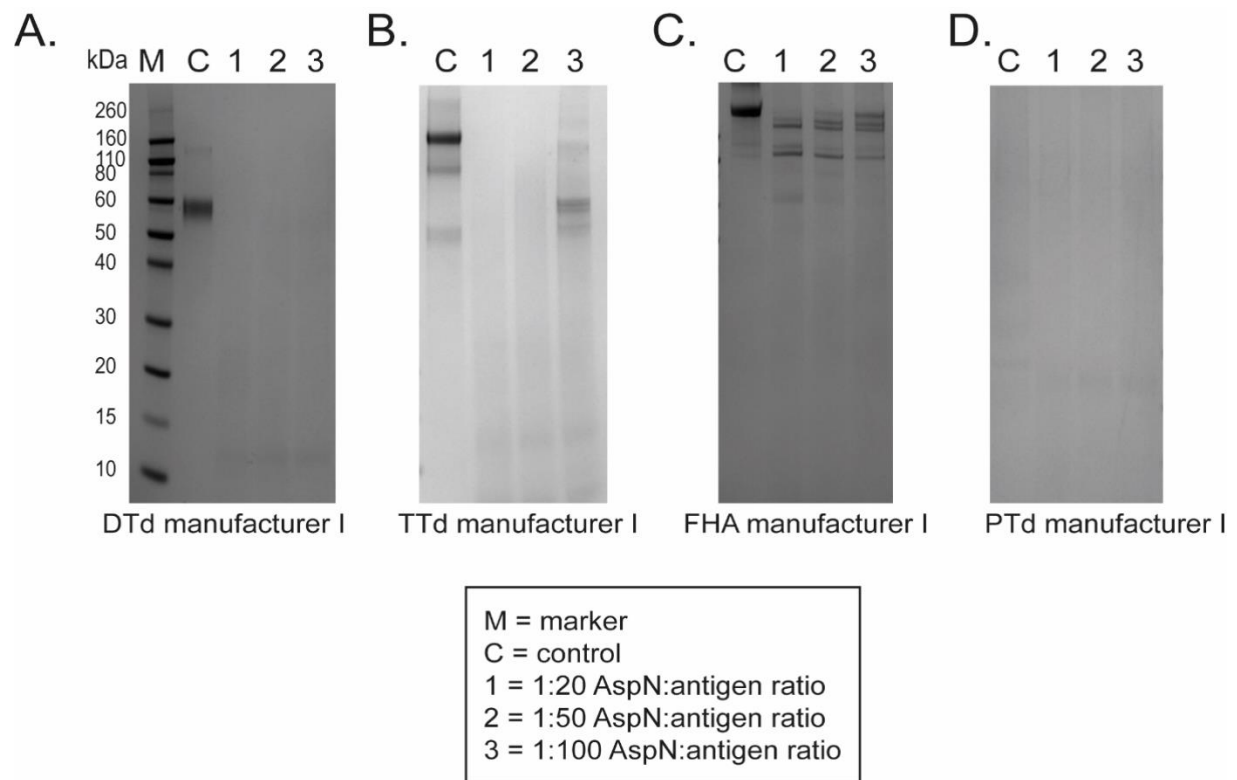


Figure S1. SDS-PAGE results digestion of DTd, TTd, FHA and PTd using different enzyme-to-substrate ratios. Non-adjuvanted samples of individual DTd (A.), TTd (B.), FHA (C.) and PTd (D.) antigens were analyzed.

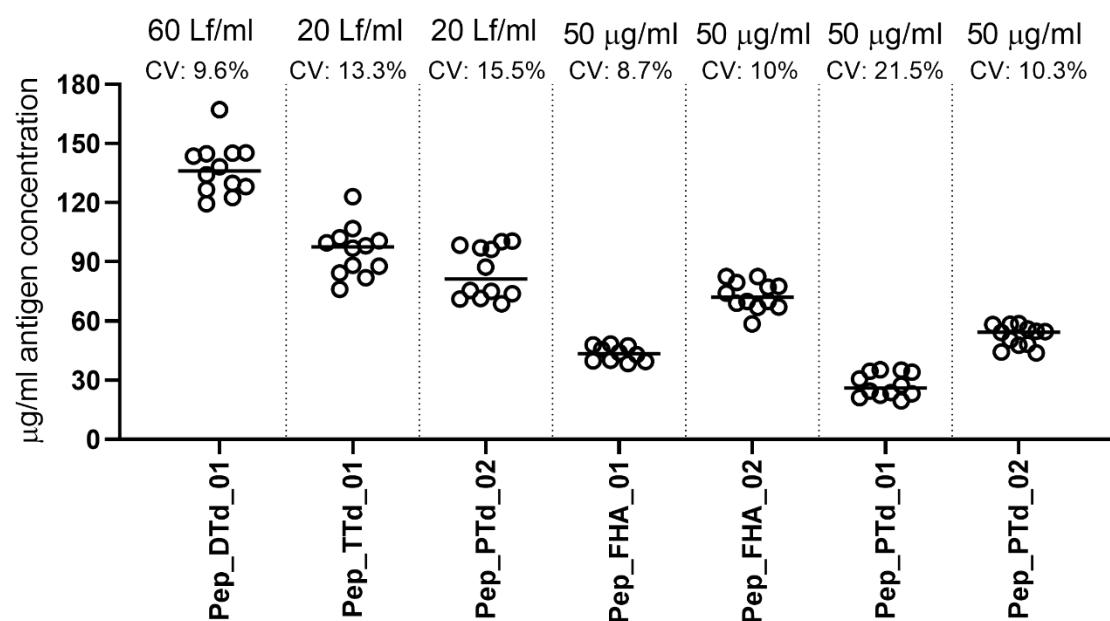


Figure S2. Quantification of antigens in a second batch of DTaP vaccine. The used signature peptides are shown on the X-axis and the antigen concentrations are plotted on the Y-axis. To determine the amount of antigen present in vaccine A batch 2, 100 pmol of each internal standard peptide was added to 100 µl vaccine and digested with 0.33 µg Asp-N (n=12 per signature peptide, except for peptide 4, n=10).