

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	GSGSGMSRKLFASIL	B1	VLALKVDNAETIKKE	C1	DKTKTKIESLKEHGP
A2	MSRKLFASILIGALL	B2	VDNAETIKKELGLSL	C2	KIESLKEHGPINKNM
A3	FASILIGALLGIGAP	B3	TIKKELGLSLTEPLM	C3	KEHGPIKNKMSESPN
A4	IGALLGIGAPPSAHA	B4	LGLSLTEPLMEQVGT	C4	IKNNKMSESPNKTVSE
A5	GIGAPPSAHAAGADDV	B5	TEPLMEQVGTTEEFIK	C5	SESPNKTSEEKAKQ
A6	PSAHAGADDVVDSSK	B6	EQVGTEEFIKRGFGD	C6	KTVSEEKAKQYLEEF
A7	GADDVVDSSKSFVME	B7	EEFIKRFGDGASRVV	C7	EAKAQYLEEFHQTA
A8	VDSSKSFVMENFSSY	B8	RFGDGASRVVLSLPF	C8	YLEEFHQTALEHPEL
A9	SFVMENFSSYHGTPK	B9	ASRVVLSLPFAEGSS	C9	HQTALEHPELSELKT
A10	NFSSYHGTPKGYVDS	B10	LSLPFAEGSSVEYI	C10	EHPSELKTVGTN
A11	HGTKGPGYVDSIQKG	B11	AEGSSSVEYINNWEQ	C11	SELKTVGTNPVFA
A12	GYVDSIQKGIQKPKS	B12	SVEYINNWEQAKALS	C12	VTGTPVFA
A13	IQKGIQKPKSGTQGN	B13	NNWEQAKALSVELEI	C13	PVFA
A14	QKPKSGTQGNYDDD	B14	AKALSVELEINFETR	C14	ANYAAWAVNV
A15	GTQGNYDDDWKGFYS	B15	VELEINFETRGKRGQ	C15	WAVNV
A16	YDDDWKGFYSTDNKY	B16	NFETRGKRGQDAMYE	C16	AQVIDSETADNLEKT
A17	KGFYSTDNKYDAAGY	B17	GKRGQDAMYEYMAQA	C17	SETADNLEKTTAALS
A18	TDNKYDAAGYSDVNE	B18	DAMYEYMAQACAGNR	C18	NLEKTTAALSILPGI
A19	DAAGYSDVNENPLSG	B19	YMAQACAGNRVRSV	C19	TAALSLPGIGSVMG
A20	SVDNENPLSGKAGGV	B20	CAGNRVRRSGVSSLS	C20	ILPGIGSVMGIADGA
A21	NPLSGKAGGVVKVTY	B21	VRRSGSSLSCINLD	C21	GSVMGIADGA
A22	KAGGVVKVTYPGLTK	B22	GSSLSCINLDWDVIR	C22	IADGA
A23	VKVTYPGLTKVLALK	B23	CINLDWDVIRDKTKT	C23	VHHNT
A24	PGLTKVLALKVDNAE	B24	WDVIRDKTKTIESL	C24	EEIVAAQSTALSSLMV
				F1	
D1	QSIALSSLMVAQAIP	E1	SVNGRKIRMRCRAID	F2	
D2	SSLMVAQAIPLVGEL	E2	KIRMRCRAIDGDVT	F3	KEVPALTAVETGATN
D3	AQAIPLVGELVDIGF	E3	CRAIDGDVTFCRPKS	F4	
D4	LVGELVDIGFAAYNF	E4	GDVTFCRPKSPVYVG	F5	GYPKDGNFNNLDRI
D5	VDIGFAAYNFVESII	E5	CRPKSPVYVGNGVHA	F6	
D6	AAYNFVESIINLFQV	E6	PVYVGNGVHANLHVA	F7	YDYDVPDYAGYPYDV
D7	VESIINLFQVHVNSY	E7	NGVHANLHVAFHRS	F8	
D8	NLFQVHVNSYNRPAY	E8	NLHVAFHRSSEKIH	F9	QEVRKYFCV
D9	VHNSYNRPAYSPGHK	E9	FHRSSSEKIHNEIS	F10	
D10	NRPAYSPGHKTQPFL	E10	SEKIHSNEISSDSIG	F11	KEVPALTAVETGATN
D11	SPGHKTQPFLHDGYA	E11	SNEISSLDSIGVLGYQ	F12	
D12	TQPFLHDGYAVSWNT	E12	SDSIGVLGYQKTVDH	F13	GYPKDGNFNNLDRI
D13	HDGYAVSWNTVEDSI	E13	VLGYQKTVDHTKVNS	F14	
D14	VSWNTVEDSIIRTGF	E14	KTVDHTKVNSKLSLF	F15	YDYDVPDYAGYPYDV
D15	VEDSIIRTGFQGESG	E15	TKVNSKLSLFFEIKS	F16	
D16	IRTGFQGESGHDIKI	E16	KLSLFFEIKSGSGSG	F17	QEVRKYFCV
D17	QGESGHDIKITAENT	E17		F18	
D18	HDIKITAENTPLPIA	E18		F19	
D19	TAENTPLPIAGVLLP	E19		F20	
D20	PLPIAGVLLPTIPGK	E20		F21	
D21	GVLLPTIPGKLDVNK	E21		F22	
D22	TIPGKLDVNKS	E22		F23	
D23	LDVNKS	E23		F24	
D24	SKTHISVNGRKIRM	E24			

Figure S1: Alignment of the primary structure of DTx [(1-*C. diphtheriae* (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-MSRKLFASIL	IGALLGIGAP	PSAHAGADDV	VDSSKSFVME	NFSSYHGTKP	GYVDSIQKGI	QKPKSGTQGN	YDDDWKGFY
2-MNRKLFASIL	IGALLGIGTP	LSAHASADDV	VDSSKSFVME	NFSSYHGTKP	GYVDSIQKGI	QKPKSGTQGN	YDDDWKGFY
3-MNRKLFALTL	IGALLGIGAP	LSAHSADDV	VDSSKSFVME	NFSSYHGTKP	GYVDSIQKGI	QKPKSGTQGN	YDDDWKGFY
-----CB/TX-1-----CB/TX-2-----CB/TX-3-----CB/TX-4-----							
90	100	110	120	130	140	150	160
1-TDNKYDAAGY	SVDNENPLSG	KAGGVVKVTV	PGLTKVLA	VDNAETIKKE	LGLSLTEPLM	EQVGTEEFIK	RFGDGASRV
2-TDNKYDAAGY	SVDNENPLSG	KAGGVVKVTV	PGLTKILALK	VDNAETIKKE	LGLSLTEPLM	EQVGSEEFIK	RFGDGASRV
3-TDNKYDAAGY	SVDNENPLSG	KAGGVVKVTV	PGLTKILALK	VDNAETIKKE	LGLSLTEPLM	EQVGSEEFIK	RFGDGASRV
-----CB/TX-5-----CB/TX-6-----							
170	180	190	200	210	220	230	240
1-LSLPFAEGSS	SVEYINNWEQ	AKALSVELEI	NFETRKGKRGQ	DAMYEYMAQA	CAGNRVRRSV	GSSLSCINLD	WDVIRDKT
2-LSLPFAEGSS	SVEYINNWEQ	AKALSVELEI	NFETRKGKRGQ	DAMYEYMAQS	CAGNRVRRSV	GNSSSCINLD	WDAIRDKT
3-LSLPFAEGSS	SVEYINNWEQ	AKALSVELEI	NFENRGKRGQ	DAMYEYMAQS	CAGNRIRRSV	SNSSSCLNLD	WDAIRDKT
-----CB/TX-8-----CB/TX-9-----							
250	260	270	280	290	300	310	320
1-KIESLKEHGP	IKNMKMSESPN	KTVSEEKAKQ	YLEEFHQ	EHPELSELKT	VTGTNPVFA	ANYAAWVN	AQVIDSETAD
2-KIESLKEHGP	IKNMKMSESPN	KAVSEEKAKQ	YLEEFHQ	EHPELSELKT	VTGTNSVFA	ANYAAWVN	AQVIDSETAD
3-KIESLKENG	IKNMKMSESPN	KTVSEEKAKQ	YLEEFHQ	EHPELSELKT	VAETNSVFA	ANYAAWVN	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	EEIVAQ	SSLMVQA	LVGELVDIG	AAYNFVESI	NLFQVVHNS
2-NLEKTTAALS	ILPGIGSVMG	IADGAVHHNT	EEIVAQ	SSLMVQA	LVGELVDIG	AAYNFVESI	NLFQVVHNS
3-NLEKTTAALS	ILPGIGSVMG	IADGAVHHNT	EEIVAQ	SSLMIAQ	LVGELVDIG	AAYNFVESI	NLFQVHNS
-----CB/TX-14-----CB/TX-15-----							
410	420	430	440	450	460	470	480
1-NRPAYSPGHK	TQPFLHDGYA	VSWNTVEDSI	IRTGFQGESG	HDIKITAENT	PLPIAGVLLP	TIPGKLDVN	SKTHISVN
2-NRPAYSPGHK	TQPFLHDGYA	VSWNTVEDSI	IQTGFQGESG	HDIKITAENT	PLPIAGVLLP	TIPGKLDVN	SKTHISVN
3-NRPTYSPGHK	TQPFLHDGYA	VSWNTVEDSI	IKTGFQGESG	HDIKITAENT	PLPIAGVLLP	TITGKLDVN	SKTHISVN
-----CB/TX-16-----CB/TX-17-----CB/TX-18-----							
490	500	510	520	530	540	550	560
1-KIRMRCRAID	GDVTFCRPKS	PVYVGNGVHA	NLHVAFHRS	SEKIHSNEIS	SDSIGVLYQ	KTVDH	KLSLFFEIKS
2-KIRMRCRAID	GDVTFCRPKS	PVYVGNGVHA	NLHVAFHRS	SEKIHSNEIS	SDSIGVLYQ	KIVDH	KLSLFFEIKS
3-KIRMQCRAID	GNVTFCRPKS	PVYVGKGVHA	NLHVAFHRS	SEKIQSNEIL	SDSIGILGYQ	KIVDH	KLSLFFEIKS
-----CB/TX-19-----CB/TX-20-----							