

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	IQKGIQKPKSGTQGN	B13	NNWEQAKALSVELEI	C13	PVFAGANYAAWAVNV
A14	QKPKSGTQGNYDDDW	B14	AKALSVELEINFETR	C14	ANYAAWAVNVAQVID
A15	GTQGNYDDDWKGFYS	B15	VELEINFETRGRGQ	C15	WAVNVAQVIDSETAD
A16	YDDDWKGFYSTDNKY	B16	NFETRGRGQDAMYE	C16	AQVIDSETADNLEKT
A17	KGFYSTDNKYDAAGY	B17	GKRGQDAMYEYMAQA	C17	SETADNLEKTTAALS
A18	TDNKYDAAGYSVDNE	B18	DAMYEYMAQACAGNR	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	AAYNFVESIINFVSI	E5	CRPKSPVYVGNGVHA	F5	GYPKDGNFNNLDRI
D6	AAYNFVESIINFVSI	E6	PVYVGNGVHANLHVA	F6	
D7	VESIINFVSIINLFQV	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	GYPKDGNFNNLDRI
D14	VSWNTVEDSIIRTGF	E14	KTVDH TKVNSKLSLF	F14	
D15	VEDSIIRTGFQGESG	E15	TKVNSKLSLFFFEIKS	F15	YDYDVPDYAGYPYDV
D16	IRTGFQGESGHDIKI	E16	KLSLFFFEIKSGSGSG	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 S1: 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	NFSSYHGTP	GYVDSIQKGI	QKPKSGTQGN	YDDDWKGFYS
2-MNRKL	FASIL	IGALLGIGTP	LSAHASADDV	VDSSKSFVME	NFSSYHGTP	GYVDSIQKGI	QKPKSGTQGN	YDDDWKGFYS
3-MNRKL	FALIL	IGALLGIGAP	LSAHASVDDV	VDSSKSFVME	NFSSYHGTP	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	NFETRGRKRGQ	DAMYEYMAQA	CAGNRVRRSV	GSSLSCLNLD	WDVIRDKTKT	
2-LSLPFAEGSS	SVEYINNWEQ	AKALSVELEI	NFETRGRKRGQ	DAMYEYMAQS	CAGNRVRRSV	GNSSSCLNLD	WDAIRDKTKT	
3-LSLPFAEGSS	SVEYINNWEQ	AKALSVELEI	NFENRGRKRGQ	DAMYEYMAQS	CAGNRIRRSV	SNSSSCLNLD	WDAIRDKTKA	
-----CB/Tx-8-----CB/Tx-9-----CB/Tx-10-----CB/Tx-11								
	250	260	270	280	290	300	310	320
1-KIESLKEHGP	IKNKMSESPN	KTVSEEKAKQ	YLEEFHQ TAL	EHPSELSELT	VTGTNPVFAG	ANYA AWA VNV	AQVIDSETAD	
2-KIESLKEHGP	IKNKMSESPN	KAVSEEKAKQ	YLEEFHQ TAL	EHPSELSELT	VTGTNSVFAG	ANYA AWA VNV	AQVIDSETAD	
3-KIESLKENG P	IKNKMSESPN	KTVSEEKAKQ	YLEEFHQ TAL	EHPSELSELT	VAETNSVFAG	ANYA AWA VNV	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	NLFQVVIHNSY	
-----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-NRPYSPGHK	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	GDVTF CRPKS	PVYVGNGVHA	NLHVAFHRSS	SEKIHSNEIS	SDSIGVLGYQ	KTV DHTK VNS	KL SLFF EIKS	
2-KIRMRCAID	GDVTF CRPKS	PVYVGNGVHA	NLHVAFHRSS	SEKIHSNEIS	SDSIGVLGYQ	KIV DHTK VNS	KL SLFF EIKS	
3-KIRMQCAID	GNVTF CRPKS	PVYVGKGVHA	NLHVAFHRSN	SEKIQSNEIL	SDSIGILGYQ	KIV DHTK VNS	KL SLFF EIKS	
-----CB/Tx-19-----CB/Tx-20-----CB/Tx-21								