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Product Data Sheet

CODE	NAME
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SPG06	Polyclonal antibodies (rabbit) raised against <i>C. difficile</i> TcdB ₂₁₋₂₄
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DETAILS	
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Description	Polyclonal antibodies (rabbit) to TcdB ₂₁₋₂₄ (Appendix 1)
Host	Rabbit
Isotype	IgG
Class	Polyclonal
Type	Antibody
Immunogen	rTcdB ₂₁₋₂₄ fragment of the cell-binding domain of <i>C. difficile</i> toxin B

TESTED APPLICATIONS	DILUTION*
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ELISA	1:1000 – 1:4000
Western Blot	1:1000 – 1:4000

* Suggested working dilutions are given as a guide only. The user is recommended to titrate the product for use in their own experiment using appropriate negative and positive controls.

FORM INFORMATION	
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Form	Liquid
Volume	250 µl
Concentration	500 µg at 2 mg/ml
Purity	Protein A purified
Storage buffer	PBS, pH 7,4
Preservative	0.05 % sodium azide
Precaution of use	This product contains sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage comments	The antibody is stable at 2-8°C for up to 6 months. For long term storage, aliquot the antibody and store at -20°C or below. Avoid repeated freeze-thaw cycles.

Quality control results

Antibodies: rabbit anti- TcdB₂₁₋₂₄ polyclonal antibody (SPG06)

1) Assay: Indirect ELISA

Coated antigens: A: TcdB₂₁₋₂₄
B: ovalbumin (negative control)

Coating concentration: 1 µg/ml

Volume per well: 50µl

Coating buffer: Phosphate Buffered Saline, pH 7.4

Secondary antibody: anti-Rabbit IgG (A9169, Sigma), dilution 1:8000

Table 1. ELISA results for anti- TcdB₂₁₋₂₄ polyclonal antibody

Dilution	A	B
1:1000	1.432	0.043
1:2000	1.472	0.045
1:4000	1.190	0.046
1:8000	0.803	0.044
1:16000	0.458	0.043
1:32000	0.271	0.045
1:64000	0.161	0.050
1:128000	0.108	0.048
1:256000	0.085	0.045
1:512000	0.063	0.048
blank	0.049	0.046
Titer:	1:128000	<1:1000

Starting dilution: 1:1000 (equivalent to 2 µg/ml)

The titer is the highest dilution with S/B (Signal/Blank) ≥ 2.1

2) Assay: Western blotting

Tested antigens: 1 500ng *C. difficile* toxin B toxoid (ENZO Life Sciences)
2 500 ng ovalbumin (negative control)

Primary antibody: anti-TcdB₂₁₋₂₄ polyclonal antibody (SPG06), dilution 1:1000

Secondary antibody: anti-Rabbit IgG (A9169, Sigma), dilution 1:5000

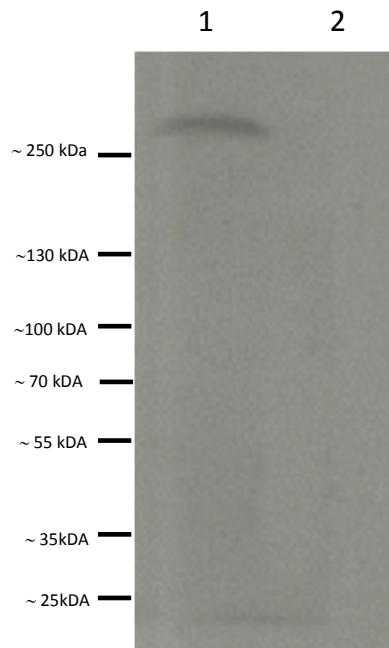


Figure 1. Western blot for toxin B toxoid (from *C. difficile*)

Lane 1: toxin B toxoid from (500ng) (270 kDa)

Lane 2: ovalbumin (500ng, negative control)

Appendix 1

Toxin B (TcdB)



TcdB₂₁₋₂₄ is a fragment of the cell-binding domain of *C. difficile* toxin B and carries repeats 21-24 [1, 2].

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      r21                      r22
MRTGLISFENNNYYFNENGE MQFGYINIEDKMFYFGEDGDMQIGVFNTPDGFKYFAHQN
      r23                      r24
TLDENFEGESINYTGWLDLDEKRYFTDEYIAATGSVIIDGEEYFDPDTAQLVISE-C terminus
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rTcdB₂₁₋₂₄ was expressed in *E. coli* as a C-terminal poly-histidine tagged protein and purified using AKTA chromatography.

[1] Barroso LA, Wang SZ, Phelps CJ, Johnson JL, Wilkins TD. Nucleotide sequence of *Clostridium difficile* toxin B gene. *Nucleic Acids Res.* 1990;18:4004.

[2] von Eichel-Streiber C, Laufenberg-Feldmann R, Sartingen S, Schulze J, Sauerborn M. Comparative sequence analysis of the *Clostridium difficile* toxins A and B. *Mol Gen Genet.* 1992;233:260-8.