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Product Data Sheet

CODE	NAME
SPG05	Polyclonal antibodies (rabbit) raised against recombinant TcdA ₂₆₋₃₉

DETAILS	
Description	Polyclonal antibodies (rabbit) to rTcdA ₂₆₋₃₉ (Appendix 1).
Host	Rabbit
Isotype	IgG
Class	Polyclonal
Type	Antibody
Tested species reactivity	Bacteria
Immunogen	TcdA ₂₆₋₃₉ fragment of the cell-binding domain of <i>C. difficile</i> toxin A

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

* 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	
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-TcdA₂₆₋₃₉ polyclonal antibody (SPG05)

1) Assay: Indirect ELISA

Coated antigens: A: rTcdA₂₆₋₃₉ fragment
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-TcdA₂₆₋₃₉ polyclonal antibody

Dilution	A	B
1:1000	1.189	0.045
1:2000	0.759	0.045
1:4000	0.481	0.045
1:8000	0.275	0.045
1:16000	0.163	0.045
1:32000	0.108	0.047
1:64000	0.077	0.045
1:128000	0.061	0.044
1:256000	0.054	0.048
1:512000	0.051	0.046
blank	0.048	0.051
Titer:	1:32000	<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: 1 500 ng *C. difficile* toxin A toxoid (ENZO Life Sciences)
2 500 ng ovalbumin (negative control)

Primary antibody: anti-TcdA₂₆₋₃₉ polyclonal antibody (SPG05), dilution 1:1000

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

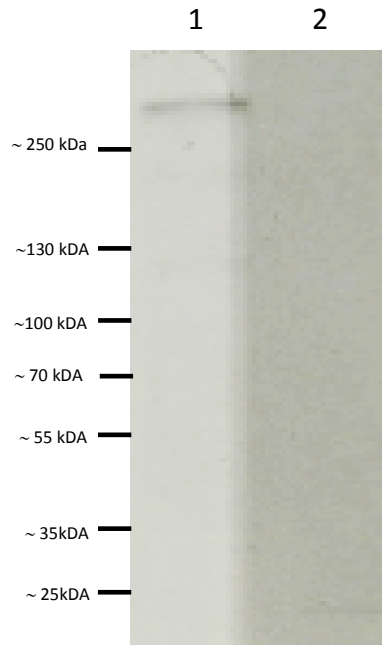
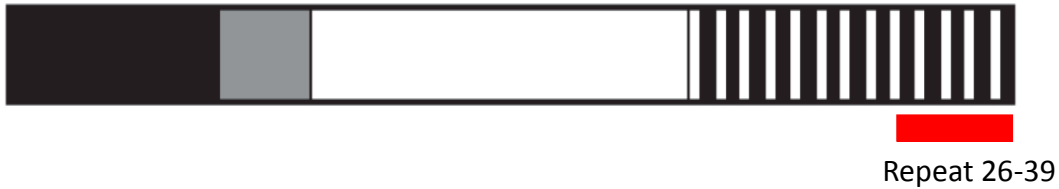


Figure 1. Western blot for toxin A toxoid from (*C. difficile*)

Lane 1: toxin A toxoid from (*C. difficile*) (308 kDa)

Lane 2: ovalbumin (negative control)

Toxin A (TcdA)



TcdA₂₆₋₃₉ is a fragment of the carboxy-terminal cell-binding domain of *C. difficile* toxin A [1-3].

STGYTSINGKHFYFNTDGIMQIGVFKGPNDFEYFAPANTDANNIEGQAILYQNKFLTLNGKKYYFGSDSKAVTGLRTIDGKK
YYFNTNTAVAVTGWQTINGKKYYFNTNTSIASGYTIISGKHFYFNTDGIMQIGVFKGPDGFEYFAPANTDANNIEGQAIRY
QNRFLYLHDNIYYFGNNSKAATGWVTIDGNRYFEPNTAMGANGYKTIDNKNFYFRNGLPQIGVFKGSNGFEYFAPANTD
ANNIEGQAIRYQNRFLHLLGKIYYFGNNSKAVTGWQTINGKVVYFMPDTAMAAAGGLFEIDGVIYFFGVDGVKAP

rTcdA₂₆₋₃₉ was expressed in *E. coli* as a C-terminal poly-histidine tagged protein and purified using AKTA chromatography.

[1] Permponpattana P, Hong HA, Phetcharaburanin J, Huang JM, Cook J, Fairweather NF, et al. Immunization with *Bacillus* spores expressing toxin A peptide repeats protects against infection with *Clostridium difficile* strains producing toxins A and B. *Infection and immunity*. 2011;79:2295-302.

[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.

[3] Dove CH, Wang SZ, Price SB, Phelps CJ, Lyerly DM, Wilkins TD, et al. Molecular characterization of the *Clostridium difficile* toxin A gene. *Infect Immun*. 1990;58:480-8.