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## Product Data Sheet

| CODE  | NAME   |
|-------|--|
| SPG04 | Monoclonal antibodies (mouse) against CotE of <i>Clostridium difficile</i> |

### DETAILS

|                               |   |
|-------------------------------|---|
| Description                   | Monoclonal antibodies against intervening peptide separating the chitinase and peroxiredoxin domains of CotE of <i>Clostridium difficile</i> [1].   |
| The antigen peptide sequence: | DNYNDEEVSKKIDNTCSWKKEHTKNIENECNCEHEHHDYLNKALDCKQEH<br>KTDIKDDCNHEKKHTKNTNKVHNSKQDKFKDKSCDEMNFNYDKDESCDKINSSYN<br>KEDSSYEDFYKHNYKNYDYTSEKNTKKIAMKTLKDSKLVLPQITDPYNPIVENANC<br>PDINPIVAEYVLGN |
| Host                          | Mouse   |
| Isotype                       | IgG   |
| Class                         | Monoclonal  |
| Type                          | Antibody  |

| TESTED APPLICATIONS | DILUTION*       |
|---------------------|-----------------|
| ELISA               | 1:4000 – 1:8000 |
| Western Blot        | 1:4000 – 1:8000 |

\* 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               | 50 µl  |
| Concentration        | 100 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  |

Storage comments freeze / thaw cycles.  
The antibody is stable at 2-8°C for up to 1 month. For long term storage, aliquot the antibody and store at -20°C or below. Avoid repeated freeze-thaw cycles.

### Quality control results

Antibodies: mouse anti-CotE monoclonal antibody (SPG04)

#### 1) Assay: Indirect ELISA

Coated antigens: A: CotE  
B: CotD (negative control)

Coating concentration: 1 µg/ml

Volume per well: 50µl

Coating buffer: Carbonate buffer, pH 9.6

Secondary antibody: anti-Mouse IgG (P0447, Dako Denmark), dilution 1:2000

*Table 1. ELISA results for anti-CotE monoclonal antibody*

| <b>Dilution</b> | <b>A</b>  | <b>B</b> |
|-----------------|-----------|----------|
| 1:1000          | 1.313     | 0.058    |
| 1:2000          | 1.332     | 0.054    |
| 1:4000          | 1.310     | 0.053    |
| 1:8000          | 1.204     | 0.051    |
| 1:16000         | 1.164     | 0.054    |
| 1:32000         | 0.988     | 0.054    |
| 1:64000         | 0.804     | 0.051    |
| 1:128000        | 0.547     | 0.054    |
| 1:256000        | 0.338     | 0.056    |
| 1:512000        | 0.212     | 0.056    |
| 1:1024000       | 0.145     | 0.057    |
| 1:2048000       | 0.113     | 0.054    |
| blank           | 0.067     | 0.0585   |
| Titer:          | 1:1024000 | <1:1000  |

The titer is the highest dilution with S/B (Signal/Blank)  $\geq 2.1$

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

## 2) Assay: Western blotting

Tested antigens:       1       spore coat extract of *C. difficile* 630  
                          2       spore coat extract of *B. subtilis* (negative control)

Primary antibody:     mouse anti-CotE monoclonal antibody (SPG04), dilution 1:8000

Secondary antibody:  anti-Mouse IgG (P0447, Dako Denmark), dilution 1:4000

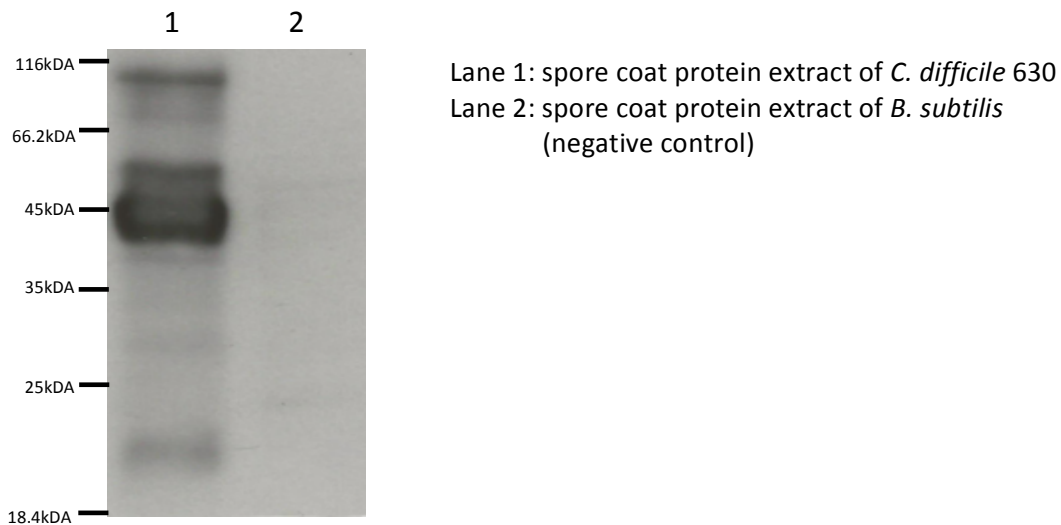


Figure 1. Western blot for spore coat proteins

### Note:

CotE CotE is an 81 kDa protein but when extracted from *difficile* spores is often fractured into species of 40-50 kDa depending upon the extraction procedure used [1]. In the figure shown CotE species of ~ 81 kDa, as well as 40-50 kDa. species are shown.

### Reference:

1. Permpoonpattana, P., et al., *Surface layers of Clostridium difficile endospores*. J Bacteriol, 2011. 193(23): p. 6461-70.