SporeGen Ltd Bourne Laboratories Royal Holloway, University of London Egham, Surrey TW20 0EX, UK

Tel: +44-(0)1784-443760 Mob: +44 (0)7733-425024 Email: info@sporegen.com www.sporegen.com

9SporeGen

Product Data Sheet

| CODE | NAME |
|---|---|
| SPG02 | Polyclonal antibodies (rabbit) raised against purified spores of <i>Clostridium difficile</i> strain 630 (ribotype 012) |
| DETAILS | |
| Description Host Isotype Class Type Tested species reactivity Immunogen | Polyclonal antibodies to <i>Clostridium difficile</i> 630 spore antigen Rabbit IgG Polyclonal Antibody Bacteria Spores of <i>Clostridium difficile</i> 630 (ribotype 012) |
| TESTED APPLICATIONS | DILUTION* |
| ELISA Western Blot | 1:1000 – 1:2000 1:1000 – 1:2000 |

* 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 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: rabbit anti-*C. difficile* 630 spore polyclonal antibody (SPG02)

1) Assay: Indirect ELISA

| Coated antigens: | A: | C. difficile 630 spores | |
|------------------|----|-------------------------|--|
|------------------|----|-------------------------|--|

B: *B. subtilis* spores (negative control)

Coating concentration: 10⁸ spores/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-C. difficile 630 spore polyclonal and | ibody |
|---|-------|

| Dilution | Α | В |
|----------|---------|---------|
| 1:1000 | 1.303 | 0.132 |
| 1:2000 | 1.034 | 0.116 |
| 1:4000 | 0.832 | 0.117 |
| 1:8000 | 0.670 | 0.115 |
| 1:16000 | 0.484 | 0.130 |
| 1:32000 | 0.348 | 0.131 |
| 1:64000 | 0.286 | 0.147 |
| 1:128000 | 0.246 | 0.138 |
| 1:256000 | 0.218 | 0.132 |
| blank | 0.203 | 0.103 |
| Titer: | 1:16000 | <1:1000 |

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

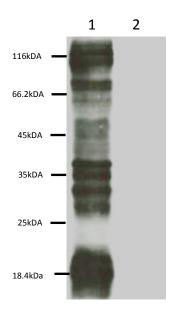
The titer is the highest dilution with S/B (Signal/Blank)≥2.1

2) Assay: Western blotting

| Tested antigens: | 1 | spore coat extract of C. difficile 630 |
|------------------|---|--|
|------------------|---|--|

2 spore coat extract of *B. subtilis* (negative control)

Primary antibody:rabbit anti-C. difficile 630 spore polyclonal antibody (SPG02), dilution 1:1000Secondary antibody:anti-Rabbit IgG (A9169, Sigma), dilution 1:5000



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

Figure 1. Western blot for spore coat proteins