

Langford Locks Kidlington Oxfordshire OX5 1LH

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Antibody Datasheet

Name: Mouse Anti SARS-CoV-2 Spike (S1) RBD Antibody (DH6)

Product Code: MAB12444-100 / MAB12444-500

Batch #:

Date of Manufacture:

Product Description: Purified monoclonal antibody specific for SARS-CoV-2. Antibody is neutralising

against SARS-CoV-2, but not against SARS-CoV-1 or MERS-CoV.

Clone Number: DH6.G6.A12.C11

Isotype: IgG1 Kappa

Amount: 0.1 mg / 0.5 mg

Concentration: 1.0 mg/ml

Presentation: Liquid

Buffer: Phosphate Buffered Saline, pH 7.4, Filter Sterile

Immunogen: SARS-CoV-2 Spike subunit 1 (S1), REC31882 (aa 1-223)

Purification: Protein G

Specificity: SARS-CoV-2 receptor binding domain (RBD) for Wuhan-Hu-1, UK (Alpha), South African (Beta) Brazilian (Gamma) and Indian variants. Antibody also binds to SARS-CoV-2 mutant spike proteins REC31899 (D614G, S477N), REC31900 (D614G, L84I, N439K), REC31901 (D614G, G485R), REC31902 (D614G, E484K) and REC31903 (D614G, V445I, H655Y, E583D). No cross-reactivity observed in ELISA with SARS-CoV-2 subunit 2 (S2) or spike subunit 1 (S1) proteins from SARS-CoV, MERS-CoV, HCoV-NL63, HCoV-OC43, HCoV-229E and HCoV-HKU1.

Applications: ELISA, NTRL.

Application Notes: Working dilution must be determined by the user. Suggested starting ranges are 0.01- $0.1 \mu g/ml$ for ELISA.

Matched Pair: Suitable for use as capture or detection antibody in ELISA assays. Capture antibody with MAB12446 as detection antibody. Detection antibody with MAB12422 or MAB12446 as capture antibody (see data below).

Usage Guidelines

Short Term Storage: +4°C Long Term Storage: -20°C

Storage Guidelines: Avoid repeat freeze-thaw cycles





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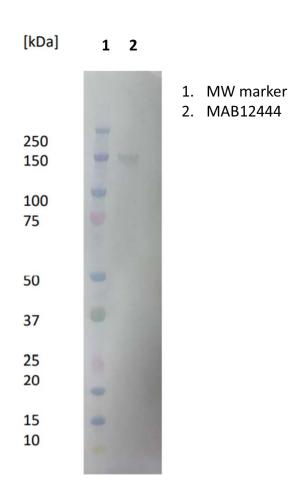
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Western Blot: 10µl (0.02mg/ml) of SARS-CoV-2 spike S1 protein (REC31806) separated on a Novex 4-12% Bis-Tris gel, alongside a Kaleidoscope marker (BioRad). Primary antibody added at 1 mg/ml (1:1000) and Goat anti mouse secondary antibody (PAB21441HRP) added at 1mg/ml (1:2000).

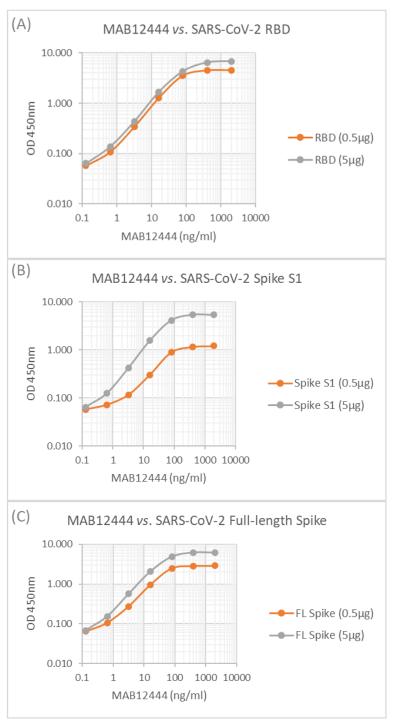




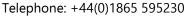


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Antigen-down ELISA: ELISA showing binding of MAB12444 to (A) immobilised SARS-CoV-2 receptor binding domain (RBD) protein (REC31882) (B) Spike subunit 1 (S1) (REC31806) and (C) full-length spike protein (REC31868).

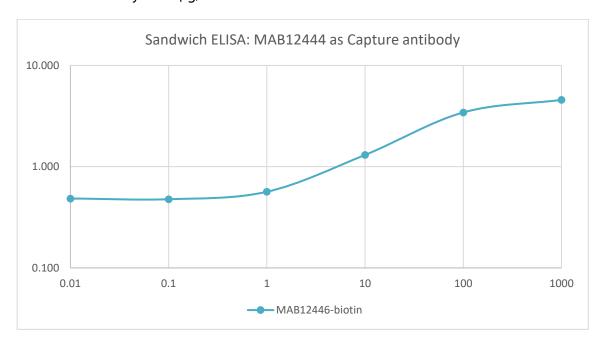




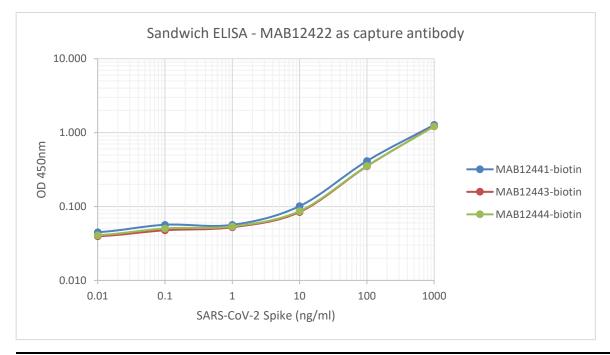




Sandwich ELISA: MAB12444 as capture antibody. SARS-CoV-2 full-length Spike (REC31868) was the capture analyte. Plates were coated with 5µg/ml of MAB12444. Spike protein was added at varying concentrations from 1µg/ml to 0.001ng/ml. Plates were incubated with biotin-labelled MAB12446 detection antibody at 0.9µg/ml.



Sandwich ELISA: MAB12444 as detection antibody. SARS-CoV-2 full-length Spike (REC31868) was the capture analyte. Plates were coated with 5µg/ml of MAB12422. Spike protein was added at varying concentrations from 1µg/ml to 0.001ng/ml. Plates were incubated with biotin-labelled detection antibodies at 1µg/ml.

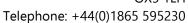




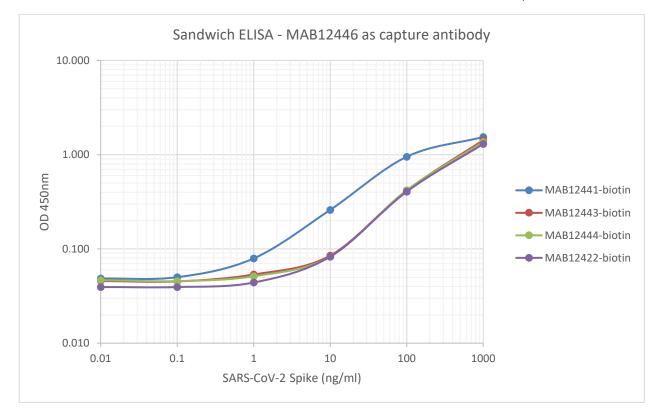


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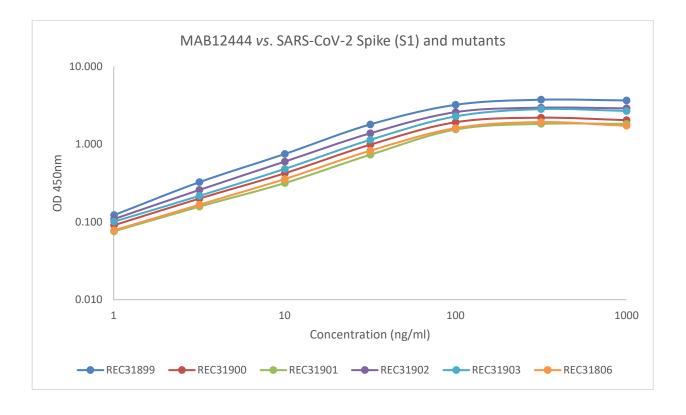
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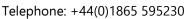




ELISA: Binding of antibody to SARS-CoV-2 spike subunit 1 (S1) protein (REC31806) and mutant S1 proteins.



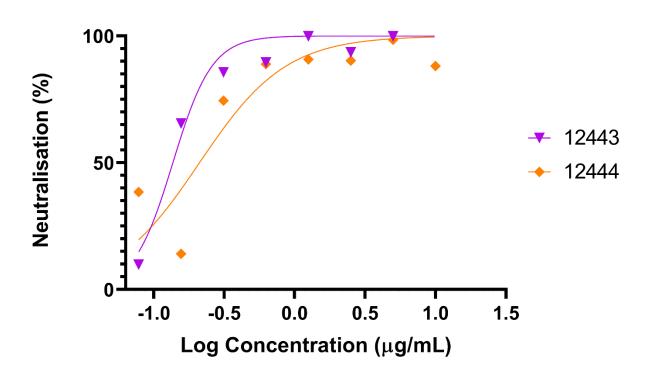






Neutralisation Assay: Neutralisation assays of monoclonal antibodies (MAB12443 and MAB12444) were carried out against lentiviral pseudotypes expressing the SARS-CoV-2, SARS-CoV-1 or MERS-CoV Spike glycoproteins on their surface. Pseudotype micro-neutralisation (pMN) assays were carried out by Dr Diego Cantoni, Dr Martin Mayora-Neto and Dr Nigel Temperton at the Viral Pseudotype Unit, Medway School of Pharmacy, University of Kent.

SARS CoV 2 Neutralisation



mAb	IC50 (µg/mL)
12443	0.136
12444	0.211



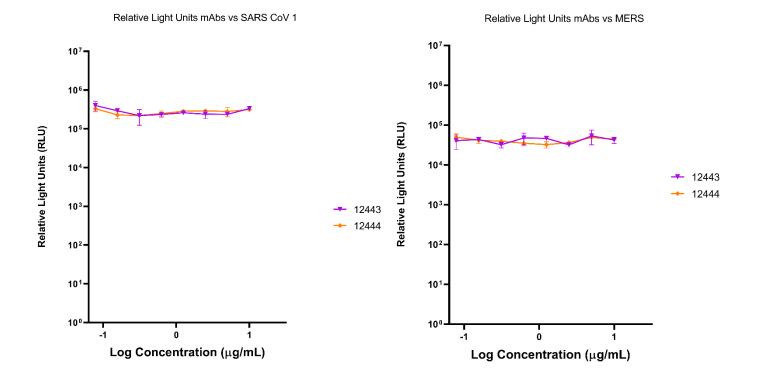


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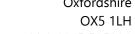
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SARS-CoV-1 and MERS-CoV Neutralisation Assays

Neutralisation was not observed for SARS-CoV-1 or MERS-CoV.



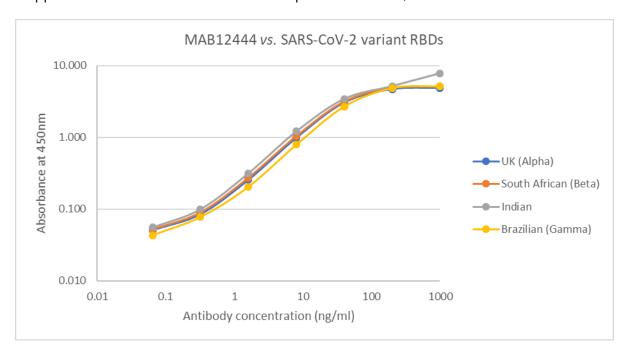


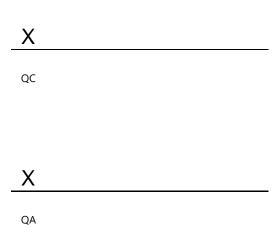




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Direct ELISA: Plate was coated with 100µl of variant RBD proteins (UK, REC31946; South African, REC31945; Indian, REC31971; Brazilian, REC31961), at 1µg/ml and then incubated with 100µl MAB12444 antibody, diluted from 1000ng/ml to 0.064ng/ml. Diluted secondary IgG HRP antibody (100µl at 1:10,000) was then added. 100µl of TMB substrate (M0701A) was added in all wells and the reaction stopped after 10 min. with 1M HCl and the plate read at 405/450nm.





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