

DESCRIPTION

Species Reactivity	HCoV-229E
Specificity	Detects Human Coronavirus HCoV-229E in direct ELISAs.
Source	Monoclonal Mouse IgG _{2B} Clone # 1045017
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Human embryonic kidney cell HEK293-derived HCoV-229E Spike RBD Ser292-Asp453 Accession # P15423.1
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

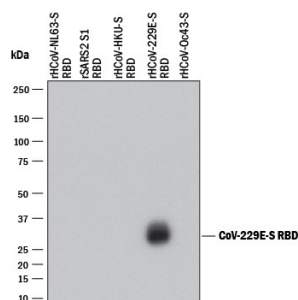
APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. [General Protocols](#) are available in the Technical Information section on our website.

	Recommended Concentration	Sample
Western Blot	1 µg/mL	Recombinant HCoV-229E-S RBD
Immunocytochemistry	8-25 µg/mL	Immersion fixed CHO Chinese hamster ovary cell line transfected with HCoV-229E
Simple Western	20 µg/mL	Recombinant HCoV-229E-S RBD

DATA

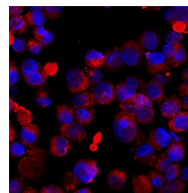
Western Blot



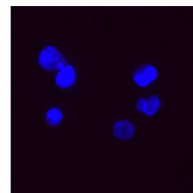
Detection of Spike RBD by Western Blot.

Western blot shows recombinant HCoV-229E-S RBD. PVDF membrane was probed with 1 µg/mL of Mouse Anti-HCoV-HKU1 Spike RBD Monoclonal Antibody (Catalog # MAB10938) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for Spike RBD at approximately 30 kDa (as indicated). This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

Immunocytochemistry



Transfected CHO cells

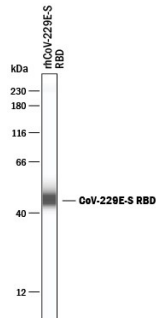


Wild type CHO cells

Spike RBD in CHO Chinese hamster ovary cell line transfected with HCoV-229E.

Spike RBD was detected in immersion fixed CHO Chinese hamster ovary cell line transfected with HCoV-229E (positive staining) and CHO Chinese hamster ovary cell line (non-transfected, negative staining) using Mouse Anti-HCoV-HKU1 Spike RBD Monoclonal Antibody (Catalog # MAB10938) at 8 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cell cytoplasm. Staining was performed using our protocol for Fluorescent ICC Staining of Non-adherent Cells.

Simple Western



Detection of SARS-CoV-2 Spike RBD by Simple Western™.

Simple Western lane view recombinant HCoV-229E-S RBD, loaded at 0.2 mg/mL. A specific band was detected for Spike RBD at approximately 47 kDa (as indicated) using 20 µg/mL of Mouse Anti-HCoV-229E Human Coronavirus Spike RBD Monoclonal Antibody (Catalog # MAB10938). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

HCoV-229E belongs to a family of viruses known as coronaviruses that are commonly comprised of a large plus-strand RNA genome and four structural proteins: Spike protein (S), Envelope protein (E), Membrane protein (M), and Nucleocapsid protein (N). HCoV-229E is a member of the alpha-coronavirus family and was discovered in 1966 (1, 2). Other well-known human coronaviruses include three viruses that cause relatively mild respiratory disease: HCoV-NL63, HCoV-HKU1 and HCoV-OC43, plus three viruses that caused the Severe Acute Respiratory Syndrome (SARS-CoV), the Middle East Respirator Syndrome (MERS-CoV), and the global pandemic Covid-19 (SARS-CoV2). HCoV-229E Spike Protein (S Protein) is a glycoprotein that mediates membrane fusion and viral entry. As with most coronaviruses, proteolytic cleavage of the S protein generates two distinct peptides, S1 and S2 subunits. The S1 subunit is focused on attachment of the protein to the host receptor while the S2 subunit is involved with cell fusion. Although HCoV-229E S protein shares high homology (56%) with HCoV-NL63, it does not employ Angiotensin-Converting Enzyme 2 (ACE2) as the receptor like HCoV-NL63. Instead, HCoV-229E engages CD13 (aminopeptidase N) for cellular entry and replication (3). The receptor binding domain (RBD) of HCoV-229E is solely responsible for receptor binding through three extended receptor binding loops (4).

References:

1. Hamre, D. and J.J. Procknow (1966) Proc. Soc. Exp. Biol. Med. **121**:190.
2. Van der Hoek, L. *et al.* (2004) Nat. Med. **10**:368.
3. Yeager, C.L. *et al.* (1992) Nature **357**:420.
4. Wong, A.H.M. *et al.* (2017) Nat. Commun. **8**:1735.