

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human 53BP1 in direct ELISAs.
Source	Recombinant Monoclonal Rabbit IgG Clone # 1285C
Purification	Protein A or G purified from cell culture supernatant
Immunogen	<i>E. coli</i> -derived recombinant human 53BP1 Ala1614-His1972 Accession # Q12888
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
Knockout Validated	2 µg/mL	See Below
Western Blot	2 µg/mL	See Below
Immunocytochemistry	3-25 µg/mL	See Below
Immunohistochemistry	1-25 µg/mL	See Below

DATA

Knockout Validated

Western Blot Shows Human 53BP1 Specificity Using Knockout Cell Line.
Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and 53BP1 knockout HeLa cell line (KO). PVDF membrane was probed with 2 µg/mL of Rabbit Anti-Human 53BP1 Monoclonal Antibody (Catalog # MAB18772) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for 53BP1 at approximately 350 kDa (as indicated) in the parental HeLa cell line, but is not detectable in the knockout HeLa cell line. GAPDH (Catalog # MAB5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Western Blot

Detection of Human 53BP1 by Western Blot. Western blot shows lysates of HEK293 human embryonic kidney cell line and ZR-75 human breast cancer cell line. PVDF membrane was probed with 2 µg/mL of Rabbit Anti-Human 53BP1 Monoclonal Antibody (Catalog # MAB18772) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for 53BP1 at approximately 350 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry

53BP1 in HeLa Human Cell Line. 53BP1 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Rabbit Anti-Human 53BP1 Monoclonal Antibody (Catalog # MAB18772) at 3 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). Specific staining was localized to nuclei. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry

53BP1 in Human Cervical Cancer Tissue. 53BP1 was detected in immersion fixed paraffin-embedded sections of human cervical cancer tissue using Rabbit Anti-Human 53BP1 Monoclonal Antibody (Catalog # MAB18772) at 1 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to nuclei. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

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	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <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

The p53-binding protein 1 (53BP1) localizes to sites of damaged DNA and is phosphorylated by the ATM checkpoint kinase. 53BP1 is required for phosphorylation of other downstream ATM substrates, such as p53 and SMC-1, and therefore aids in nucleating a DNA damage response protein complex. Over aa 1614-1972, human 53BP1 shares 97% aa sequence identity with rat and mouse 53BP1.