

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects recombinant mouse RNF168 and recombinant human RNF168 in direct ELISAs and Western blots.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant mouse RNF168 Asn423-Arg565 Accession # Q80XJ2
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 as a 0.2 µm filtered solution in PBS.

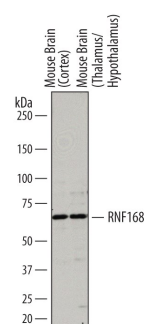
APPLICATIONS

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	0.5 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below

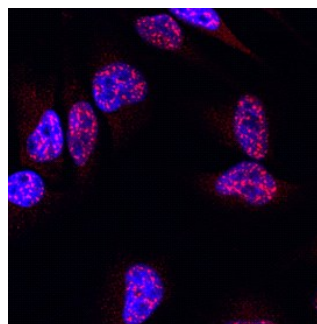
DATA

Western Blot



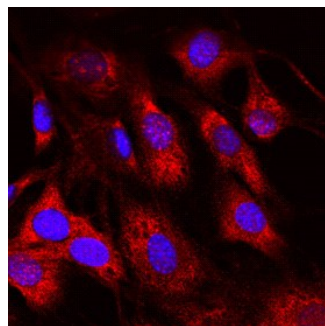
Detection of Mouse RNF168 by Western Blot. Western blot shows lysates of mouse brain (cortex) tissue and mouse brain (thalamus/hypothalamus) tissue. PVDF membrane was probed with 0.5 µg/mL of Sheep Anti-Mouse RNF168 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7217) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for RNF168 at approximately 65 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



RNF168 in HeLa Human Cell Line. RNF168 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Sheep Anti-Mouse RNF168 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7217) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Immunocytochemistry



RNF168 in NIH3T3 Mouse Cell Line. RNF168 was detected in immersion fixed NIH3T3 mouse embryonic fibroblast cell line using Sheep Anti-Mouse RNF168 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7217) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.2 mg/mL.
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

RNF168 (RING [really interesting new gene] finger protein 168; also E3 ubiquitin-protein ligase RNF168) is a 65 kDa (predicted) member of the RNF168 family of proteins. It is ubiquitously expressed, and serves as an E3 ubiquitin ligase. Following DNA damage, RNF8 is recruited to DNA double-strand breaks by phosphoMDC1. Here, RNF8 first monoubiquitinates histone H2A, and then promotes RNF168 recruitment. RNF168 now acts as an additional ubiquitinase, promoting multiple ubiquitinations plus the recruitment of 53BP1, a scaffold protein that holds DNA damage response elements. Mouse RNF168 is 565 amino acids (aa) in length. It contains one Zn-finger/RING domain (aa 16-55) plus two ubiquitin-interacting MIU motifs (aa 168-191 and 438-461). There are three potential Ser/Thr phosphorylation sites. Two potential isoform variants are reported. One shows an alternative start site at Met20, while a second possesses a two aa extension at the N-terminus coupled to a 13 aa substitution for aa 343-565. Over aa 423-565, mouse RNF168 shares 79% and 65.5% aa sequence identity with rat and human RNF168, respectively. Over aa 423-466, mouse RNF168 shares 98% aa sequence identity with human RNF168.