

## Affinity-Purified Goat Anti-human/mouse/rat RNF2 Antibody

### ORDERING INFORMATION

**Catalog Number:** AF6065

**Lot Number:** CDOQ01

**Size:** 100 µg

**Storage:** -20° C

**Specificity:** human, mouse and rat RNF2

**Immunogen:** *E. coli*-derived recombinant human RNF2 (aa 147 - 228)

**Ig Type:** goat IgG

**Application:** Western blot

### Background

RNF2 (RING [really interesting gene] finger protein 2/1B; also BAP-1, HIP-2 interacting protein 3 and DinG) is a 37 kDa member of the ring finger domain family of proteins. It is expressed in embryonic tissue, and serves as an E3 ubiquitin ligase within a polycomb complex that acts on histone H2A. In effect, RNF2 contributes to gene repression by blocking RNA pol II advance along gene promoters. RNF2 forms homodimers, and interacts with over a dozen proteins. Human RNF2 is 336 amino acids (aa) in length contains one Zn-finger domain (aa 51 - 91) and two phosphorylation sites at Ser168 and Ser203. There is one potential isoform that shows a deletion of aa 84 - 155. Over aa 147 - 228, human RNF2 shares 99% aa identity with mouse RNF2.

### Preparation

Produced in goat immunized with purified, *E. coli*-derived, recombinant human RNF2 (rhRNF2; aa 147 - 228; Accession # Q99496). Human RNF2 specific IgG was purified by affinity chromatography.

### Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

### Reconstitution

Reconstitute the antibody in 100 µL PBS containing 0.02% NaN<sub>3</sub>. The antibody concentration will be 1.0 mg/mL.

### Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

### Specificity

The antibody detects endogenous human, mouse and rat RNF2 at ~40 kDa by Western blot.

### Application

**Western blot** - An antibody concentration of 1.0 µg/mL is recommended.

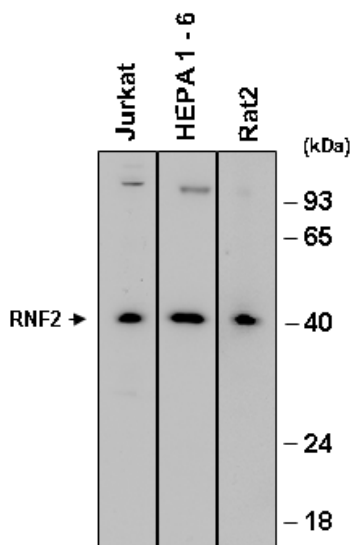
#### Protocols for Immunoblotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	5% nonfat dry milk	2% nonfat dry milk
0.15 M NaCl	in Blotting Buffer	in Blotting Buffer
0.1% Tween® 20	Adjust pH to 7.4	Adjust pH to 7.4

- Transfer the electrophoresed proteins to an Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
- Incubate the membrane overnight at 4° C in antibody solution containing 1.0 µg/mL goat anti-human/mouse/rat RNF2.
- Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
- Incubate the membrane for 1 hour at room temperature in Antibody Solution containing a 1:2,000 dilution of HRP-conjugated Donkey anti-goat IgG (R&D Systems, Catalog # HAF016).
- Wash the membrane for 1 hour with 5 or more changes of blotting buffer.
- Detect with chemiluminescent reagents.

**Cell lysates for Western blottings** - To prepare total cell lysates, cells are solubilized in hot 2X SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF and bromophenyl blue) at  $2 \times 10^6$  -  $1 \times 10^7$  cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with 3 - 4 bursts of 5 - 10 second each. Samples are diluted with 1X SDS sample buffer to the desired concentration.

**Optimal dilutions should be determined by each laboratory for each application.**



### Detection of RNF2 with AF6065.

Lysates from human Jurkat, mouse Hepa 1 - 6 and rat Rat2 cells were resolved by SDS-PAGE, transferred to Immobilon-P membrane and immunoblotted with 1.0 µg/mL goat anti-RNF2 as described in *Protocols for Immunoblotting*.