

## Affinity-Purified Goat Anti-human Bcl-10

### ORDERING INFORMATION

**Catalog Number:** AF2889

**Lot Number:** UYY01

**Size:** 100 µg

**Storage:** -20° C

**Specificity:** human Bcl-10

**Immunogen:** *E. coli* derived recombinant human Bcl-10 (aa 1 - 192)

**Ig Type:** goat IgG

**Application:** Western blot

### Preparation

Goat antibodies were raised against purified, *E. coli*-derived, recombinant human Bcl-10 (also known as B-cell CLL/lymphoma 10), amino acids 1-192, (GenBank accession number NM\_003921). Polyclonal antibody was affinity-purified on a column derivatized with recombinant human Bcl-10.

### 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 aliquoted 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 is known to react with endogenous levels of human Bcl-10 on Western blots.

### Application

**Western blot** - An antibody concentration of 0.5 µg/mL is recommended.

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

### Protocols for Immunoblotting:

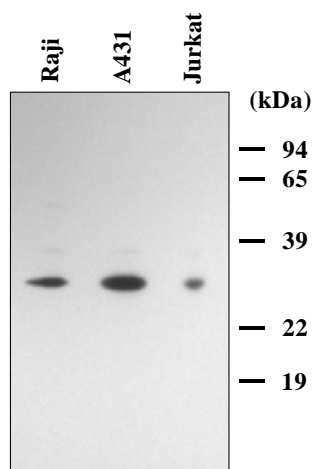
#### Western blotting

| <u>Blotting Buffer</u>     | <u>Blocking Solution</u>              | <u>Antibody Solution</u>              |
|----------------------------|---------------------------------------|---------------------------------------|
| 25 mM Tris, pH 7.4         | 2% nonfat dry milk in Blotting Buffer | 1% nonfat dry milk in Blotting Buffer |
| 0.15 M NaCl                | Adjust pH to 7.4                      | Adjust pH to 7.4                      |
| 0.1% Tween <sup>®</sup> 20 |                                       |                                       |

1. Transfer the electrophoresed proteins to an Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 4° C in antibody solution containing 0.5 µg/mL goat anti-Bcl-10.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane for 1 hour at room temperature in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated donkey anti-goat IgG (R&D Systems, Catalog # HAF109).
5. Wash the membrane for 1 hour with 5 or more changes of blotting buffer.
6. Detect with Western Glo Chemiluminescent detection reagents (R&D Systems, Catalog # AR004) or equivalent.

**Cell lysates for Western blots:** 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.

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### Detection of Bcl-10 with AF2889.

Lysates from human Raji, A431 and Jurkat cells were resolved by SDS-PAGE, transferred to Immobilon-P membrane and immunoblotted with 0.5 µg/mL goat anti-Bcl-10 as described in *Protocols for Immunoblotting*. A 30 second exposure to film is shown.