

**ORDERING INFORMATION****Catalog Number:** BAF2584**Lot Number:** UTF01**Size:** 50 µg**Storage:** -20° C**Reconstitution:** sterile 0.1% BSA in TBS**Specificity:** mouse HSP10/EPF**Immunogen:** *E. coli*-derived mHSP10/EPF  
(aa 2-102)**Ig Type:** goat IgG**Application:** Western blot**Background**

The heat shock proteins (HSPs) are a highly conserved family of stress response proteins. HSPs function primarily as molecular chaperones, facilitating the folding of other cellular proteins, preventing protein aggregation, or targeting improperly folded proteins to specific degradative pathways. Heat Shock Protein 10 (HSP10), also known as chaperonin 10 (Cpn10), HSPE1, and Early Pregnancy Factor (EPF), is a 10 kDa heat shock protein that functions within the cell as a molecular chaperone and is associated with HSP60 in the mitochondria. Early pregnancy factor (EPF) has been identified as an extracellular homologue of HSP10.

**Preparation**

Goat antibodies were raised against purified, *E. coli*-derived full-length recombinant mouse HSP10/EPF, (aa 2 - 102; GenBank Accession # NM\_008303). Polyclonal antibody was affinity-purified on a column derivatized with the recombinant protein and further purified by isolating the IgG fraction.

**Formulation**

Lyophilized from a 0.2 µm-filtered solution in phosphate-buffered saline (PBS) containing 50 µg of bovine serum albumin (BSA) per 1 µg of antibody.

**Reconstitution**

Reconstitute with sterile Tris-buffered saline pH 7.3 (20 mM Trizma base, 150 mM NaCl) containing 0.1% BSA. If 1 mL of buffer is used, the antibody concentration will be 50 µg/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**

This antibody detects endogenous levels of mouse HSP10/EPF in Western blots.

**Applications**

**Western Blot** - An antibody concentration of 0.25 µg/mL is recommended.

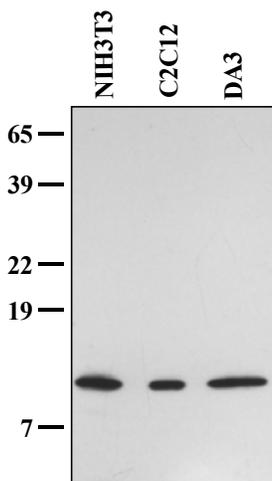
**Optimal dilutions should be determined by the individual laboratory.**

**Protocols for Immunoblotting**

<u>Blotting Buffer</u>	<u>Blocking Solution</u>	<u>Antibody Solution</u>
25 mM Tris, pH 7.4	5% nonfat dry milk in Blotting Buffer	3% BSA in Blotting Buffer
0.15 M NaCl	Adjust pH to 7.4	Adjust pH to 7.4
0.1% Tween 20		

1. Transfer the electrophoresed proteins to 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 0.25 µg/mL anti-mouse HSP10/EPF.
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 3 % BSA in blotting buffer containing a 1:200 - 1:2,000 dilution of HRP-conjugated Streptavidin (R&D Systems, Catalog # DY998).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with WesternGlo Chemiluminescent Detection Reagent (R&D Systems, Catalog # AR004) or equivalent.

**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 a probe sonicator with 3 - 4 bursts of 5-10 seconds each. Tween is a registered trademark of ICI Americas.



**Detection of HSP10/EPF with BAF2584.** Lysates of mouse NIH-3T3, C2C12, and DA3 cells were resolved by SDS-PAGE, transferred to an Immobilon-P membrane and immunoblotted with 0.25 µg/mL anti-mouse HSP10/EPF as described in *Protocols for Immunoblotting*. A 30 second exposure to film is shown.