



#### ORDERING INFORMATION

**Catalog Number:** BAF254

**Lot Number:** ARP03

**Size:** 50 µg

**Formulation:** 0.2 µm filtered solution in PBS with BSA

**Storage:** -20° C

**Reconstitution:** sterile TBS with 0.1% BSA

**Specificity:** human ENA-78

**Immunogen:** *E. coli*-derived rhENA-78

**Ig Type:** goat IgG

**Applications:** Western blot  
ELISA detection

## ***Biotinylated Anti-human ENA-78 Antibody***

### ***Preparation***

Produced in goats immunized with purified, *E. coli*-derived, recombinant human ENA-78 (rhENA-78). Human ENA-78 specific IgG was purified by human ENA-78 affinity chromatography and then biotinylated.

### ***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 has been selected for use as a detection antibody in human ENA-78 ELISAs and western blots.

### ***Applications***

**Western Blot** - This antibody can be used at 0.1 - 0.2 µg/mL with the appropriate secondary reagents to detect human ENA-78. The detection limit for rhENA-78 is approximately 5 ng/lane under non-reducing and reducing conditions.

**ELISA** - This antibody can be used as a detection antibody in a human ENA-78 ELISA in combination with the monoclonal capture antibody (Catalog # MAB654). A general protocol is provided on the next page. Using plates coated with 100 µL/well of the capture antibody in combination with 100 µL/well of the detection antibody at 100 ng/mL, an ELISA for sample volumes of 100 µL can be obtained. To arrive at the optimal dose range for this ELISA, set up a two-fold dilution series of the protein standard starting with 1 ng/mL. In this format, less than 0.2% cross-reactivity with rhGCP-2 is observed and less than 0.05% cross-reactivity with rmGCP-2, rhGRO $\alpha$ , rrCINC-1, rmKC, rhNAP-2 and rhIL-8 is observed.

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

## ***ELISA Protocol***

### **Solutions Required**

- **Wash Buffer** - 0.05% Tween 20 in PBS, pH 7.4
- **Diluent** - 1% BSA in PBS
- **Substrate Solution** - 1:1 mixture of Color Reagent A (H<sub>2</sub>O<sub>2</sub>) and Color Reagent B (Tetramethylbenzidine) (R&D Systems, Catalog # DY999)
- **Stop Solution** - 1 M H<sub>2</sub>SO<sub>4</sub>

### **Plate Preparation**

1. Transfer 100 µL/well of the capture antibody (diluted to the appropriate concentration in PBS, use immediately) to an ELISA plate. Seal plate and incubate overnight at room temperature.
2. Aspirate each well and wash with Wash buffer, repeating the process two times for a total of 3 washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper toweling.
3. Block plates by adding 300 µL of PBS containing 1% BSA, 5% sucrose and 0.05% NaN<sub>3</sub> to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition. Alternatively, the blocking buffer can be aspirated after step 3 and the plates can be dried under a vacuum. When sealed with desiccant, the plates can be stored at 4° - 8° C for at least 2 months.

### **Assay Procedure**

1. Dilutions of unknowns and standards should be carried out in polypropylene tubes. Add 100 µL of sample or standards in an appropriate diluent, per well. Mix by gently tapping the plate frame for 1 minute. Cover with an adhesive strip and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of Plate Preparation.
3. Add 100 µL of the biotinylated detection antibody, diluted in the appropriate diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2 of Plate Preparation.
5. Add 100 µL streptavidin HRP (R&D Systems, Catalog # DY998, dilute according to the directions on the vial label) to each well. Cover the plate and incubate for 20 minutes at room temperature.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 µL of Substrate Solution to each well. Incubate for 20 - 30 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

### **Calculation of Results**

To calculate assay results, average the duplicate readings and subtract the zero standard optical density from the sample optical density. Plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log-log paper and regression analysis may be applied to the log transformation. To determine the human ENA-78 concentrations for each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding human ENA-78 concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. A standard curve should be generated for each set of samples assayed.

### **Limitations**

It is important that the diluents selected for reconstitution and for dilution of the standard reflect the environment of the samples being measured. The diluent suggested in the above protocol may be suitable for most cell culture supernate samples. Validate diluents for specific sample types prior to use.

A basic understanding of immunoassay development is required for the successful use of these reagents in immunoassays. The protocol provided is for demonstration purposes only. The type of enzyme and substrate and the concentrations of capture/detection antibodies used can be varied to create an immunoassay with a different sensitivity and dynamic range.