Version 21-04/24

User's Manual

Rat Receptor Associated Protein Immunolabeling Kit

REF **DEIA-CL004**

Σ

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

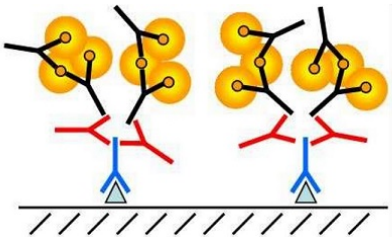
Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

General Description

This immunolabeling system allows a clean amplification of the signal enabling the detection of low amounts of antigens. The rabbit antibody (shown in red) bridges the **rat** primary antibody (shown in blue) to the **rat** monoclonal anti-HRP antibody (shown in black) previously immunologically bound to HRP. Contrary to HRP conjugation to the secondary antibody, a procedure which inactivates binding sites or enzymatic activity and therefore lowers the signal and increases background, this system results in cleaner and enhanced signals for the accurate detection of antigenic sites revealed by any **rat** antibody.



This kit is designed to be used with **rat primary antibodies**.

Reagents And Materials Provided

1. Blocking solution (normal rabbit serum (purple)
2. Diluent solution (for primary antibody and secondary rabbit anti-rat IgG's Fc antibody) (grey)
3. Rabbit anti-rat IgG's Fc antibody (red)
4. Rat monoclonal anti-HRP antibody (green)

Materials Required But Not Supplied

1. Primary antibody
2. Hydrogen peroxide
3. Oxidizable peroxidase substrate
4. Buffers
5. HRP solution

Reagent Preparation

Blocking solution: (purple)

1) Reconstitute each vial in 1 ml of distilled water
2) Working solution: make a 1:5 dilution in preferred buffer (see Note # 12). If desired, add 0.1-0.3 % of Triton X-100 to the blocking solution. See also Note #1 and Note # 4

Diluent solution: (grey)

1) Reconstitute each vial in 2 mL of distilled water

2) Working solution: make a 1:5 dilution in preferred buffer (see Note # 12). If desired, add 0.1-0.3 % of Triton X-100 to the diluent

solution. See also Note # 1 and Note # 4

Secondary antibody (rabbit antibody): (red)

1) Reconstitute each vial in 500 µL of distilled water
2) Working solution: make a 1:10 dilution using diluent solution or preferred buffer. See also Note # 1 and Note # 12

RAP: Rat Anti-HRP monoclonal antibody (for RAP/HRP complex): (green)

1) Reconstitute each vial in 500 µL of distilled water
2) Working solution: make a 1:10 dilution using diluent solution or preferred buffer. See also Note # 1 and Note # 12

Assay Procedure

Staining procedures for paraffin sections:

1. Deparaffinize and hydrate tissue sections through xylenes (or other clearing agents) and series of graded ethanol.
2. Wash slides 2 times 5 minutes in buffer (see Note # 12 on buffers in Notes section below).
3. For quenching endogenous peroxidase activity (if needed), incubate sections in 0.3% hydrogen peroxide (H2O2) in water or buffer for 20-30 minutes.
4. Wash slides 2 times 5 minutes in buffer.
5. Incubate sections in blocking solution for 30 minutes.
6. Blot excess solution from sections.
7. Incubate sections in primary antibody (rat) diluted in diluent solution or preferred buffer. Incubation time and primary antibody dilutions should be optimized by the user.
8. Wash slides 3 times 5-10 minutes in buffer.
9. Incubate sections with secondary antibody (prepared accordingly to instructions above) for 30-60 minutes.
10. Prepare the RAP/HRP complex as follows: prepare **RAP** antibody working solution to which HRP is added (final concentration of HRP should be 5 µg/mL); gently shake for **30 minutes prior to use** on sections (**incubate the complex RAP+HRP during incubation time of the 3 washes at step # 11**).
11. Wash slides 3 times 10 minutes in buffer.
12. Incubate sections in the RAP/HRP complex for 30-60 minutes.
13. Wash slides 3 times 5-10 minutes in buffer.
14. Incubate sections in peroxidase substrate solution and let develop until desired staining intensity is obtained.
15. Wash slides 3 times 5-10 minutes in buffer or water.
16. If desired, perform counterstaining.
17. Dehydrate sections in a series of graded ethanol, clear with xylene or other clearing agent and mount slides with mounting medium. Let slides dry completely before microscope examination.

Staining procedures for frozen sections:

1. Air dry sections.
2. Fix sections with acetone, ethanol or methanol for 5-15 minutes.
3. Wash slides 3-4 times 5-10 minutes in buffer.
4. If quenching endogenous peroxidase activity is needed proceed as in step # 3 of the staining procedure.
5. Follow the procedure for paraffin sections from step # 4 to # 17.

Special considerations for free floating thick sections (35-50 µm)

1. Longer incubation times might be required for each antibody and should be determined by the user (incubation times can go up to 36 hours at 4°C for certain primary antibodies).
2. Secondary antibody can be incubated with the sample for longer times (e.g. 2 hours to overnight at 4°C, to be optimized by user).
3. The RAP/HRP complex can be incubated with the sample overnight at 4°C with gentle agitation (make sure evaporation does not occur).
4. If using DAB for electron microscopy staining, the concentration of the DAB can be lowered slightly and contrast can be increased by adding cobalt and nickel to the solution.

Performance Characteristics

? Increased detection efficiency

? Cleaner stainings (lower background)

? Saving of the primary antibody

? High tissue penetrability

? No endogenous biotin blocking needed

? Longer shelf life due to lyophilization of the reagents

Precautions

1. It is recommended that the reconstituted solutions be aliquoted and stored frozen for subsequent uses.
2. The lyophilized reagents can be stored at 4°C or -20°C for long-term storage.
3. Use only freshly prepared buffers to avoid bacterial contamination.
4. Blocking solution and diluent solution can be diluted further if desired with preferred buffer to 1:10 instead of 1:5 if non-specific staining (background) is low. 0.1-0.3 % of Triton X-100 can be added to the blocking solution or diluent solution.
5. If desired, use liquid blocker pen (hydrophobic pen) to encircle the tissue sections to confine the flow of reagents to a defined area. This will allow using minimal amounts of reagents and saving of precious antibodies. Use after step # 2 of staining procedure.
6. The staining procedure can be carried out in a humid chamber to avoid evaporation of reagents.
7. If antigen retrieval is needed, perform after step # 2.
8. The development time (step # 14) may vary greatly depending on the substrate used, the levels of antigen in the samples, the concentrations of antibodies used and the desired staining intensity.

9. In the case of sections of neuronal tissues which are usually thicker than other tissues, longer incubation times may be required for optimal staining.
10. Solutions containing inhibitors of peroxidase activity (e.g. sodium azide) should not be used to dilute the peroxidase substrate.
11. If background staining is observed, rabbit secondary antibody and RAP (rat anti-HRP) antibody can be diluted further.
12. Buffers: PBS or PBS plus Triton X-100 (0.1%-0.3%) are commonly used buffers for immunostaining procedures.