Instruction Manual
VDPro® Rabies FA Reagent
CAT.NO. RC-RAB-11

! New version Notification: Read instruction manual before use.

1. Introduction

The **VDPro® Rabies FA reagent** is indirect fluorescent antibody (FA) test reagent. The FA reagent contains pre-diluted monoclonal antibody against nucleocapsid (NC) protein of rabies virus and FITC conjugated anti-mouse conjugate. This reagent is intended for the detection of rabies NC proteins in culture and in acetone-fixed brain and submaxillary tissues of infected animals.

2. Principle

The **VDPro® Rabies FA reagent** uses a pre-diluted monoclonal antibody against the rabies nucleocapsid protein to detect the virus in infected tissue. The antibody is incubated with rabies-infected tissue and will bind to rabies antigen present. Unbound antibody is removed by washing and the antigen-antibody complex is visualized by following reaction of FITC anti-mouse conjugate. Rabies protein in infected cells will fluorescence bright apple-green, background will be stained bright red cause of Evans blue counterstained.

3. Reagents Contents

Anti-Rabies monoclonal antibody(Mab)	----------	**8㎖**
Rabies Mab clone 2C6	above 0.1ug/ml	
FITC Anti-mouse Conjugate	----------	**8㎖**
FITC Goat Anti-mouse IgG	1/100	
Evans blue counter stain	less than 0.01%	
10X Washing Buffer	----------	**120㎖**
FA Mounting Fluid	----------	**3㎖**

4. Materials and instruments required (not supplied)

1) Slide glass and cover slip
2) Cryocut Microtome
3) Fluorescent microscope (FITC filter: excitation peak = 490nm, emission peak = 515nm)
4) Humid chamber
5) Coplin staining jars
6) Homogenizer (aerosol-tight)
7) Normal and infected mouse brain suspensions
8) Positive and negative control slides

5. REAGENTS PREPARATION

1X Washing Buffer
Mix 90㎖ of distilled water with 10㎖ of 10X Washing Buffer.
Keep in refrigerate and use within 2 weeks

PRECUTION

1) For *in vitro* use only.
2) Do not use past expiratory date.
3) Incubate the reagent in room temperature before use and return to refrigerator after use.
4) Do not allow the reagent to dry on the slides during the staining procedure.
5) Handle all specimens, slides and materials coming in contact with them as potentially infectious. Decontaminate with 0.05% sodium hypochlorite.
6) Pooling or alteration of any reagent may cause erroneous results.
7) Acetone is extremely flammable and harmful if swallowed or inhaled. Keep away from heat, sparks, or flames. Use adequate ventilation and avoid breathing vapor.
8) Performance of the fluorescence microscope is critical in achieving satisfactory test results. Microscope objectives, bulb intensity and wattage, and filters may affect results.
9) Reagent that is visibly cloudy should not be used.

6. Sample preparation

- Fluorescent antibody testing is most often performed on brain (hippocampus Ammon's horn) and submaxillary gland specimens. Each rabies specimen should be collected so as to avoid cross-contamination of specimens and contamination of laboratory surfaces.
- Specimens to be tested should be stored at 2-8℃ if testing will be performed within 24 hours. If extended storage is required, specimens should be stored at -70℃ or colder.

7. Immunofluorescence Procedure

1) Remove the acetone-fixed Positive and Negative Control slides and tissue section slides from the freezer and allow equilibrating to room temperature.
2) Add 4-5 drops of 1X anti-rabies monoclonal antibody to slide. Care must be taken to avoid cross contamination of slides during the staining procedure.
3) Incubate 30min at room temperature (or 37℃ incubator) in humid chamber.
4) Remove excess solution from the slides by briefly rinsing with 1X Washing Buffer. Next wash the slide 2-3 times briefly in 1X Washing Buffer.
5) Add 4-5 drops of 1X FITC anti-mouse conjugate and incubate 30min at room temperature (or 37℃ incubator) in humid chamber.
6) Remove excess solution from the slides by briefly rinsing with 1X Washing solution. Next wash the slide 3 times briefly in 1X Washing Buffer.
7) Mount cover slip on slides using 2-3 drop of FA mounting fluid.
8) Examine the slide on Fluorescent microscope with filter adapted in FITC.

8. Interpretation

- Specific green fluorescent like large and small dust like inclusions indicate positive reaction.
- If non-specific reaction was shown, retest using 1:2 diluted FITC anti-mouse conjugate with PBS.
- Demonstration of non-specific staining (such as that due to certain bacteria) can be confirmed by staining with a FITC-IgG conjugate directed against an unrelated virus, such as canine distemper virus.

Note:

- Non-specific staining may occur due to non-specific binding of the reagent to leukocytes and certain types of connective tissue. These reactions are morphologically distinct from the reaction seen with rabies-infected cells.
- The intensity of fluorescence seen in any positive specimen will be a function of the microscope used including filter set and light source, dilution of the Reagent and the quality of the tissue specimen.
- This reagent will detect rabies-related lyssaviruses other than rabies. Further testing may be necessary to confirm virus type.
- Slides mounted with glycerol greater than 10% and pH less than 8.0% may be subject to fading and loss of staining intensity.

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