

BioREE

Application instructions for “BioREE” biological sample staining kit in preparation for scanning electron microscopy examination

1. Purpose

1.1. “BioREE” reagent kit is designed for the preparation of biological samples for scanning electron microscopy examination.

2. Kit specifications and functioning principle

2.1. Kit contents:

- rinsing solution, labelled red “1 – liquid for primary rinsing”, 10 mL (1 vial);
- contrast agent solution, labelled yellow “2 – contrasting by $\text{NdCl}_3+\text{NaCl}$ ”, 2 mL (1 vial);
- final rinsing solution, labelled green “3 – liquid for final rinsing”, 10 mL (1 vial);
- 6-well plate (optional).

2.2. “BioREE” kit is designed for simultaneous preparation of two samples.

NOTE: if necessary, the kit may be split into two independent parts.

2.3. Kit functioning principle.

The first rinsing removes the components of growth mediums and the liquids of tissue ground substances sorbed on the surface of the sample. The following soaking in the rare earth element solution leads to elective accumulation of the REE on cell membranes. The second rinsing removes the surplus of the staining agent.

3. Safety Precautions

3.1. The components of the kit in used concentrations are non-toxic.

4. Equipment and materials necessary for using the kit:

- forceps for carrying the samples;
- a dropper capable of picking up to 2 mL of liquid volume for carrying the solutions to the staining container;
- shaking machine capable of succussion with 3-4 mm amplitude at 4-8 Hz frequency at +37°C temperature (preferably);

- scanning electron microscope capable of detecting back-scattered electrons (BSE) is required for registering the results of staining.

5. Preparing reagents for the analysis

5.1. Reagents must be soaked for 20 minutes at room temperature, then thoroughly mixed upturning every vial.

6. Analysis procedure

6.1. Prepare appropriate amount of reagents depending on the number of samples being examined.

6.2. Put “1 – liquid for primary rinsing” into the first plate well.

6.3. Put the sample into the first plate well and rinse it thoroughly for 2 minutes.

6.4. Put “2 – contrasting by $\text{NdCl}_3 + \text{NaCl}$ ” reagent into the second plate well.

6.5. Put the sample into the second plate well.

6.6. Incubate the sample for 30 minutes at 37°C , preferably with shaking.

6.7. Put “3 – liquid for final rinsing” into the third plate well.

6.8. Put the sample into the third plate well for 5-10 seconds for rinsing.

6.9. Remove the surplus of moisture.

6.10. Place the sample on the microscope stage.

7. Kit storage and application conditions

7.1. “BioREE” kit must be stored at $+2^\circ$ to $+8^\circ\text{C}$ temperature. Storage period is 2 months. Transportation and short-term storage at RT are acceptable.

7.2. **Date of manufacture:** _____

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Upon authorization of FSBIS “RIED”, according to the patent application RU 2578977 C1.

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