

# APPLICATION INSTRUCTIONS FOR “BIOREE-B” BIOLOGICAL SAMPLE STAINING KIT IN PREPARATION FOR SCANNING ELECTRON MICROSCOPY EXAMINATION

## 1. PURPOSE

1.1. “BioREE-B” 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:

①	“1 - liquid for initial rinsing” (rinsing solution, labelled red) - 1 vial, 5 mL
②	“2 - NdCl <sub>3</sub> -based contrasting agent” (first contrast agent solution, labelled yellow) - 1 vial, 2 mL
③	“3 - liquid for first intermediate rinsing” (solution for first intermediate rinsing, labelled green) - 1 vial, 5 mL
④	“4 - Pb(CH <sub>3</sub> COO) <sub>2</sub> -based contrasting agent” (second contrast agent solution, labelled purple)- 1 vial, 2 mL
⑤	“5 - liquid for fixation concentrate dilution” (labelled orange) - 1 vial, 4,5 mL
⑥	“6 - fixation concentrate” (solution for a long-term sample fixation, labelled black) - 1 vial, 0,5 mL
⑦	“7 - liquid for final rinsing” (final rinsing solution, labelled blue) - 1 vial, 5 mL

2.2. “BioREE-B” kit is designed for a simultaneous preparation of two samples with the sample volume not exceeding 3 mm<sup>3</sup> or its carrier’s size not exceeding 10 cm<sup>2</sup>.

### 2.3. Kit functioning principle:

The initial 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 first intermediate rinsing removes surpluses of the first contrasting agent. The second contrasting solution accumulates in the areas with easily oxidizable organic compounds and replaces neodymium in simple inorganic compounds. The following rinsing removes surpluses of the second contrasting agent. If long-term storage of the sample is required or when working with pathogenic organisms, the solution for long-term sample fixation can be applied.

## 3. SAFETY PRECAUTIONS

3.1. Except the solution for long-term sample fixation, all the components of the kit are non-toxic in used concentrations.

## 4. EQUIPMENT AND MATERIALS NECESSARY FOR USING THE KIT:

- receptacles for steeping samples in the solutions
- a dropper capable of picking up to 2 mL of liquid volume for carrying the solutions to the staining container
- scanning electron microscope capable of detecting back-scattered electrons (BSE) is required for registering the staining results

## 5. PREPARING REAGENTS FOR THE ANALYSIS

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

## 6. ANALYSIS PROCEDURE

6.1. Put “1 – liquid for initial rinsing” into the sample receptacle. If the sample is adhered inside a container (e.g. a 2D cell culture in a Petri dish), it is possible to perform sample preparation by sequentially changing solutions in the container.

6.2. Thoroughly rinse the sample for 1–2 minutes by swaying the receptacle and remove the liquid.

6.3. Preventing the sample from drying, fill the receptacle with “2 –  $\text{NdCl}_3$ -based contrasting agent”

6.4. Incubate the sample for 30 minutes at room temperature.

After this stage the sample can be stored in a hermetically sealed container at  $4^\circ\text{C}$  for 7 days (e.g. when it is necessary to transport the sample).

6.5. Remove the liquid from the sample receptacle and add reagent “3 – liquid for first intermediate rinsing”, leaving half of it for the next rinsing.

6.6. Thoroughly rinse the sample by swaying the receptacle.

6.7. Remove the liquid, fill the receptacle with “4 –  $\text{Pb}(\text{CH}_3\text{COO})_2$ -based contrasting agent” reagent and incubate for 15 minutes (it is recommended that samples attached to a substrate should be immersed in the solution upside down avoiding their contact with the bottom of the container).

6.8. Place the sample into the remaining half of reagent “3 – liquid for first intermediate rinsing” (left after the implementation of paragraph 6.5).

6.9. Thoroughly rinse the sample for 0.5–1 minute by swaying the receptacle.

6.10. If long-term storage of the sample is required or when working with pathogenic organisms, the sample fixation should be performed. To achieve it, mix reagents “5 – liquid for fixation concentrate dilution” and “6 – fixation concentrate”. Pour the resulting solution onto the sample.

6.11. Place the sample into the next receptacle with “7 – liquid for final rinsing” reagent.

6.12. Rinse the sample for 5–10 seconds by swaying the receptacle.

6.13. Remove moisture surplus.

6.14. Place the sample on the microscope stage. If examination cannot be carried out immediately, transportation and short-term storage of dried at RT samples are acceptable.

## 7. KIT STORAGE AND APPLICATION CONDITIONS

7.1. “BioREE-B” kit must be stored at  $+4^\circ$  to  $+8^\circ\text{C}$  temperature. Storage period is 12 months. Transportation and short-term storage at RT are acceptable.

7.2. Manufacture date: \_\_\_\_\_

Packer: \_\_\_\_\_

**QC PASSED**

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