

MANUAL FOR BioREE-CC KIT USING FOR REVERSIBLE CONSERVATION OF BIOLOGICAL SAMPLES

1. PURPOSE

1.1. BioREE-CC reagent kit is designed for a short-time conservation of the biological samples, if its further revitalization with a minimal molecular response is being planned.

2. BioREE-CC KIT PROPERTIES AND MECHANISM OF ACTION

2.1. Contents:

1	"1 - primary flushing fluid" (red label) - 1 ampule, 5 ml
2	"2 - NdCl ₃ -based conservator" (yellow label) - 1 ampule, 2 ml
3	"3 - first washout fluid" (green label) - 1 ampule, 5 ml
4	"4 - second washout fluid" (violet label) - 1 ampule, 2 ml
5	"5 - third washout fluid" (orange label) - 1 ampule, 5 ml
6	"6 - fourth washout fluid" (black label) - 1 ampule, 5 ml
7	"7 - fifth washout fluid" (blue label) - 1 ampule, 5 ml

2.2. Mechanism of action

Primary flushing removes the components of growth medium and fluids of the main tissue substance, sorbed on a sample surface. Subsequent exposure to a rare-earth element solution causes a quick and synchronous shutdown of the calcium- and phosphorus-

dependent transport systems of a cell, with the free phosphate remnants accumulating as insoluble salts. The substances resulting from inhibition are inert and excrete from the cell within 24 hours as soon as it is back in its physiologic condition. This happens during five consequent washout stages. The last step of processing is actual cell placement into a growth medium in physiologic conditions for cultivation. For most mammalian cells they are as follows: 37°C, 5% carbon dioxide, and 100% humidity.

3. PRECAUTIONARY PRECAUTIONS WHEN APPLYING THE BioREE-CC KIT

All the kit components are non-toxic in the concentrations provided.

4. MATERIALS AND EQUIPMENT REQUIRED FOR THE BioREE-CC KIT APPLYING:

- dispenser for fluid uptake up to 5 ml;
- growth medium (if sample revitalizing required)

5. REAGENT PREPARATION

The kit is balanced for processing biological samples, isotonic conditions for which equal 0,09 NaCl equivalent. First try the preservative solution on the test sample. Processing freshwater organism samples allows diluting provided preservative solutions with deionized water up to 2.5 times in volume.

Reagents “1” and “2” are to be kept in room temperature for 20 mins and mixed thoroughly by turning over each ampule.

Attention! If a sample is adhered within the container (e.g., 2D cell culture on a Petri dish), all preservation and revitalization steps can be conducted by consequent solution change in the container.

Protocol:

5.1. Put the “1 – primary processing fluid” into a container with a sample.

5.2. Wash the sample thoroughly for 1–2 minutes and remove the fluid.

5.3. Be sure to avoid the sample drying up and pour over the “2 – NdCl₃-based conservator” reagent.

5.4. Incubate the sample for 15 minutes at room temperature, after which a molecular genetic analysis or transportation are possible.

During a long-term storage be sure to create conditions that exclude moisture evaporation from the container with preserved sample.

6. REVITALIZATION

Guaranteed revitalization is possible after keeping the sample in the preservative solution for 4 hours at room temperature or for 8 hours at +4°C.

Reagents “3”-“7” are to be kept in room temperature for 20 mins and mixed thoroughly by turning over each ampule.

Protocol:

6.1. If revitalizing is required, place the sample into solutions from “3” to “7”, consequently changing them after 30 second exposure to each.

6.2. The last step of revitalizing is placing the sample into its physiologic growth medium and optimal cultivation conditions.

7. STORAGE AND APPLYING CONDITIONS

7.1. BioREE-CC kit is to be kept at temperature from +5 to +25°C. Shelf life: 1 year.

7.2. Manufacture date: _____

Packer: _____

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