cell projects

Instructions for use of Electrobuffer Kit

STORE AT CORRECT TEMPERATURES ON ARRIVAL

Product details

Electrobuffer is an Electroporation buffer designed to optimise transfection into Eukaryotic cells. Electrobuffer has been developed to produce higher transfection efficiencies with increased cell survival rate over the use of PBS or standard tissue culture media.

Electroporation is a common method which releases a high intensity electric pulse to the cells causing temporary transient holes to form in the cell membrane, while in this state DNA and other molecules can permeate into the cell from the surrounding medium. During this process the cells suffer considerable trauma and a very high kill rate is routinely encountered.

Electrobuffer has a buffer composition that is close to the natural cytoplasmic osmalarity thereby helping to reduce the trauma affects. Other compounds have also been added to protect the membrane against peroxides/degrading agents and to improve the cells ability to survive and recover from the electroporation process.

Storage and Stability

The Electrobuffer kits are shipped at ambient temperature, on arrival **PLEASE NOTE** that some of the components should be stored according to the table below upon arrival. Electrobuffer is stable for 1 Year if stored as instructed.

Vial	Storage Condition
Solution A	Room Temperature Approx. +20°C to+22°C
Solution B	Room Temperature Approx. +20°C to+22°C
Material C	-20°C
Material D	-20°C

Kit contents.

Catalogue No: EB-101 - Trial kit

Vial	No.	Qty	Contents
Solution A	1 x	50 ml	Washing Solution
Solution B	1 x	2.5 ml	Electrobuffer 2x concentrate
Material C	1 x	5.5 mg	ATP
Material D	1 x	7.7 mg	Glutathione

One kit contains enough material to prepare 6 electroporation samples of approx. 800µl.

Catalogue No: EB-110

Vial	No.	Qty	Contents
Solution A	2 x	100 ml	Washing Solution
Solution B	4 x	2.5 ml	Electrobuffer 2x concentrate
Material C	4 x	5.5 mg	ATP
Material D	4 x	7.7 mg	Glutathione

One kit contains enough material to prepare 24 electroporation samples of approx. 800µl.

Note on the Standard protocol.

The conditions for optimal electroporation efficiency vary between different cell types. Each specific cell line or primary cell culture will have its own specific culture and media requirements. However, these differences in culture conditions are not likely to influence the protocol for electroporation with Electrobuffer.

Cell growth phase and Confluence

Cells must be growing exponentially to obtain the optimum transient expression of a reporter construct. Exponentially growing cells should be obtained by seeding the cells the day before Electroporation so that on the day of transfection the surface of the flasks or dishes has approx. 70-80 % confluence.

Cell suspension preparation

1. Harvest the cells using your normal method.
2. Wash the cells twice in serum free growing medium by centrifugation (50g for 10 minutes) to remove any contaminating trypsin and foetal calf serum.
3. Re-suspend the cells in 50 ml of solution **A** (Washing solution) at room temperature.
4. Carefully count the cells. The concentration of the cells in the electroporation medium should be carefully controlled to obtain reproducible transfection efficiencies.
5. Transfer aliquots of $5x10^6$cells in 1.5 ml minifuge tubes and centrifuge to a pellet at 50g for 10 minutes.
6. Prepare the electroporation medium by mixing solution **B** (2.5ml of 2x concentrate Electrobuffer solution) with material **C** (5.5mg ATP) and **D** (7.7mg Glutathione). Vortex well until completely dissolved.
7. For each sample, mix in a 1.5ml minifuge tube, 30 µg of purified DNA (for the first trials, then adjust the DNA concentration accordingly for subsequent experiments), 400 µl of the 2x concentrate Electrobuffer (see previous paragraph) and adjust the final volume to 800 µl with high purity water. MilliQ water or equivalent is important.
8. Re-suspend the cells in the electroporation medium and incubate at room temperature for a maximum of 3 minutes.

Electroporation procedure

1. Transfer the cell suspension into the cuvette.
2. Trigger the pulse without delay.
3. Immediately (less than 30 seconds is recommended) after the pulse transfer the cell suspension to a dish containing pre-warmed culture medium with serum.
4. Refresh the culture medium after overnight culture.
5. For transient experiment, harvest the cells between 40-48 hours after transfection.

References

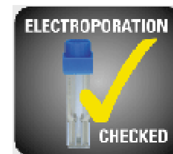
[1]. Chemomordik, L.V., Sukharev, 8.1., Popov, S.V., Pastushenko, V.F., Sorkirko, A.V., Abidor, I.G. and Chizmadzhev, Y.A. (1987) The electrical breakdown of cell and lipid membranes: the similarity of phenomenologies. Biochim. Biophys. Acta 902, 360-373

[2]. Tsong, T.Y. (1991) Electroporation of cell membranes. Biophys. J 60, 297-306

[3]. van den Hoff, M.J.B., Labruyère, W.T., Moorman, A.F.M. and Lamers, W.H. (1990) The osmolarity of the transient expression of genes. Nucl. Acids Res. 18, 6464.

Other Cell Projects Products

Electroporation Cuvettes – The Cell Projects range of HiMaX electroporation cuvettes are designed to maximise molecular electroporation and electrofusion efficiencies for Bacteria, Yeast, Insect, Plant and Mammalian cells. Each batch of cuvettes has to undergo rigorous testing at several stages during the manufacturing process for engineering tolerances, biocompatibility and sterility, prior to their being Quality tested for optimal and reproducible impedance measurements.



Safety and Use of the Electrobuffer kits

The Electrobuffer kit is intended for use by qualified professionals trained in potential laboratory hazards and good laboratory practise. If direct information is not available on any of our compounds this should not be interpreted as an indication of product safety.

This kit has been designed for research use only