Protocol 0495

GEMINI™ & ECM® 830 ELECTROPORATION PROTOCOL

Cell Line: HEK 293, HEK 293T
Application or Transfectant: DNA, pEGFP Plasmid

Cell Preparation:
1. Maintain cells in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 25 U/mL penicillin, 25 μg/mL streptomycin and 10 μg/mL gentamicin.
2. Allow cells to reach 70% confluency.
3. Harvest cells by trypsin/PBS and vigorous pipetting for 30 seconds.
4. Stop trypsinization and add complete medium supplemented with FBS.
5. Wash 5 x 10^6 cells/ml with PBS and resuspend in electroporation medium containing plasmid.

Square Wave Electroporation Settings:
(Gemini) Preset Protocol: Mammalian, 293T
(ECM 830) Voltage Mode: Low Voltage (LV)
(ECM 830) Set Voltage: 230 V
(ECM 830) Set Pulse Length: 4 msec
(ECM 830) Set Number of Pulses: 1 Pulse
Field Strength: 575 V/cm

Electroporation Procedure:
Electrode gap: 4 mm gap cuvette, Item # 45-0126
Electroporation Medium: 150 mM sucrose, 27 mM Na₂HPO₄, pH 7.5
Cell Density: 5 x 10⁶ cells/ml
Total sample Volume: 400 μl
Amount of transfectant: 10 μg plasmid DNA
Temperature: Room Temperature
Gemini Pulse: Tap the Omega icon to run load check, then with load measurement OK press the Go icon.
ECM 830 Pulse: Press Start switch to activate the automatic charging and pulse sequence.
Post Treatment: Transfer cell immediately to 10 mL of complete culture medium and place in a 37°C, 5% CO₂ incubator overnight. After 24h, harvest cells again by trypsin/PBS, wash in media and resuspend in 500 μL PBS.
Results:
>50% Percent positive transfection was measured taking the average number of GFP expressing cells from three 100-cell fields counted manually under low power with fluorescence microscopy. >65% viability was measured 24 hours after electroporation cell counts and trypan blue staining.

Reference:

Compiled by: M. Ng, BTX Technical Support, 3/8/2017