Protocol 0370

ElectroSquarePorator™

T820/830 Electroporation Protocol

Cell line: HepG2, Human Hepatocellular Carcinoma (ATCC HB 8065)

Transfectant: pGL3 (Promega SV40 promoter & enhancer, luciferase)

Cell Preparation:

Growth medium:	Grow cells in DME/F12 containing antibiotics and 10% FBS. Cells must be in good condition (exponential growth).
Trypsinization:	Wash with FBS free media, add trypsin; inactivate trypsin with 10% FBS.
Washing Procedure:	Wash cells 2x using DME/F12 media with 2.5% FBS (no antibiotics)
Cell Density:	Resuspend @ $1x10^7$ cells/ml.

Electroporation Settings:

Choose Mode:	LV Mode (99 msec/500V)
Set Voltage:	150 V
Set Pulse Length:	70 msec
Set Number of Pulses:	1
Chamber:	BTX Disposable Cuvette Model 640, 4mm gap
Desired Field Strength:	375 V/cm

Electroporation Procedure:

Sample Volume:	400 μl
DNA Concentration:	50 μg/ml, or 20 μg total
Temperature:	Room temperature (5 min. pre and post incubation)
Pulse:	Press the Start button to activate the Automatic Charge & Pulse sequence
Post Pulse Treatment:	Plate $0.4x10^6$ transfected cells into a single well of a 24 well plate. Add 1 ml of DME/F12 media with 10% FBS and incubate for 24 hours @ 37°C.

Results:

80-90 % cell viability. Luciferase activity 1,700x above background.

Reference:

Personal communication, Dr. David Pasco, National Center for the Development of Natural Products, University of Mississippi