BIO-RAD

Gene Pulser® Electroprotocols

Cell Type Other Cell Types

Species Used *Trypanosoma brucei brucei* , AnTat 1.3A, (blood- stream forms); EATRO 1125 (procyclic forms).

Molecules Electroporated DNA: plasmid constructs (containing VSG- gene, promoter*T. brucei*

Before the Pulse

Cell growth medium Cunningham's medium + 15% Fetal Calf Serum (See references in notes)

Wash solution Not given

Growth phase at harvest Mid- log phase

Pre-pulse incubation Not given

The Pulse

Electroporation Temperature Ambient temperature

Electroporation Medium Zimmerman's post-fusion medium

Cell Density 2 x 10 (7) / ml

Volume of Cells 500 μl

DNA Concentration 1 mg / ml

DNA Resuspension Buffer TES buffer

Volume of DNA 20 to 50 μl

Instruments Used Gene Pulser® apparatus

Cuvette Gap 0.4 cm

Voltage 1.5 kV

Field Strength 3.75 kV/cm

Capacitor 25 μF

Resistor (Pulse Controller) none Ω NOT RECOMMENED***(see notes)

Time Constant Not given

After the Pulse

Outgrowth Medium Cunningham's medium/Baltes Medium (procyclics) / (Bloodstream

Outgrowth Temperature 27 °C procyclics/ 37° C bloodstream

Length of Incubation 12 to 18 hours

Selection Method or Assay Used CAT assay

Electroporation Efficiency Not done

Per Cent Survival 30 to 60 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

The conditions described are not those used in my own publication, Jefferies, *et. al*. 1991*Mol. Cell. Biol.* **11:** 338-341, but were used by Clayton *et. al*., 1990 *Mol. Cell. Biol.* **10**: 3036-3047, to transfect procyclic trypanosomes.

**It is NOT RECOMMENDED to use high voltage with out the Pulse Controller.

Name of Submittor

Institution Address

Telephone Number

Fax Number

Date Submitted 3/12/90

Survey Number 191

© Bio-Rad Laboratories, 1993