***Trypanosoma brucei brucei* bloodstream form**

**(Tbb 427 90.13)**

**Transfection :**

Count the BSF cells in 3,7% formaldehyde in VPBS

Use 5-10ug of digested DNA for each electroporation

Grow 5 x 105 -1x 106 cells for each electroporation (IMDM “complet”, see below).

Spin cells @1800 rpm for 10 min at 37°C

Resuspend in 5 ml Cytomix of 37oC

Spin cells @1800 rpm for 10 min at 37°C

Remove supernatant, but leave some to resuspend the cells 300-400 ul.

In the 2 mm electroporation cuvette, add the cells and linearised DNA.

Incubate 1 min at 37°C. Electroporate at 1250 V, 25 Ohms, 50 uF

Resuspend the cells in 12 ml of IMDM complet (+ neomycin) prewarmed at 37°C.

Aliquote 500 ul in 24 well plate.

Next day, add 500 ul medium (IMDM complet + neomycin) supplemented with the selection drug 2x in each well.

**IMDM complet**

* 3.024 g Sodium Bicarbonate ( NaHCO3 , Sigma S-6014)
* 0.136 g Hypoxanthine (Sigma H-9636)
* 0.110 g Sodium pyruvate (Sigma P-3662)
* 0.039 g Thymidine (Sigma T-9250)
* 0.028 g Bathocuprone sulfate acide (Sigma B-1125).
* 1 bottle of IMDM powder for 1 liter (Iscove's Modified Dubecco's Medium , Gibco ref: 42200-014 #3011115).
* 1 liter of Aguettant water.

Mix the powders and the IMDM into a 1 liter bottle with Aguettant water qsp 1 liter.

Filter sterilise (0.22 um filter) (Nalgene) and aliquot 400 mL into bottles of 500 mL.

Store the media at + 4°C.

Before to use it :

In 400 mL of medium add :

* 1 mL of 100 mM β mercaptoethanol (Sigma M-6250 stock : 14.3 M)in water.
* 5 mL of 150 mM L-cysteine (Sigma C-1276 , 0.2364 g in 10 mL water) in water.
* 40 mL of FCS heated at 54°C 30 minutes.
* 2.5 mL 10 mg/mL Kanamycine (Sigma K-4000).

Filter sterilise (0.22 um).

Store the medium at + 4°C.

**Cytomix**: adapted from Hoff et al, 1992

stock for 500 ml

EGTA pH7.6 2 mM 0.5 M 2 ml

KCl 120 mM 1 M 60 ml

\*K2HPO4/KH2PO4 pH7.6 10 mM 0.1 M (10x) 50 ml

HEPES pH7.6 25 mM 1 M 12.5 ml

MgCl2.6H2O 5 mM 1 M 2.5 ml

Glucose (Dextrose) 0.5% 2.5 g

BSA 100 ug/mL 0.05 g

\*\*Hypoxanthine 1 mM 100x 5 ml

adjust pH to 7.6 with KOH, filter sterilise and store at 4°C.

\* Prepare 10x K2HPO4/KH2PO4 pH7.6 by mixing 8.66 ml 1 M K2HPO4 with 1.34 ml 1 M KH2PO4 in 90 ml H2O.

\*\* Hypoxantine (100x). Dissolve 0.4 g of sodium hydroxide in 100 ml of H2O, then add 1.36 g hypoxanthine.

**10x VPBS(-Sucrose-Glucose)** (Voorheis’s modified PBS):

NaCl 80 g/l

KCl 2.2 g/l

Na2HPO4 22.7 g/l

KH2PO4 4.4 g/l

Adjust pH7.4 and autoclave.

For VPBS 1x

Dilute the 10x VPBS with 15.7 g/l sucrose and 1.8 g/l glucose final concentration.

**Subculture :**

**>> The cells concentration should not be above 1.5 to 2.106/mL <<**

**>> They might not recover <<**

* over 24h :

dilute the cells to **105/mL** in a 25cm2 flask with a final volume of 5 mL (or 15 mL in a 75 cm2 flask => keep the ratio 1mL / 5cm2 for flasks)

* over the week-end :

dilute the cells to **104/mL** in a 25cm2 flask with a final volume of 5 mL (or 15 mL in a 75 cm2 flask => keep the ratio 1mL / 5cm2 for flasks)

**Defreezee stabs :**

Prepare a 24 wells plate with 4 wells of adapted pre-warmed medium (1mL/well).

Defreeze the vial and load 1 drop of the frozen stock in 1 well then 2 drops in the next well, then three drops in the following well and in the last well, load what is left in the tube.

**Freeze stabs :**

Spin down 10 ml of 5.105 cells/mL culture (800g, 10min), resuspend the pellet of cells into 1 mL of IMDM complet + 7% glycerol. Aliquots in two cryotubes. Freeze them into a Mister frosty box at -80°C. The day after put the tube into the liquid nitrogen container.