Polyoma middle T PRODUCTION FROM THE PACKAGING CELL LINE GgP+E.

Polyoma middle T PRODUCTION

• After thawing, GgP+E packaging cells are seeded l,5xl0^6 cells/T25 in DMEM supplemented with 15%
FBS HyClone.

• When cultures are confluent (usually 2 days after seeding), the cells are detached by a short incubation with the Trypsin-EDTA solution and re-seeded (4xl0^4/cm^2).

• When the cultures are confluent, the following protocol is applied:

1st day-afternoon: The GgP+E cells are seeded 2xl0^6 cells/T25

2nd day- afternoon The cells are refed with 2ml/T25 culture medium.

3rd day-morning The conditioned medium is collected, centrifuged at 1500xg for 15 min and filtered through 0.45µm.
After the filtration, the supernatant can be used immediately or aliquoted and stored at -80°C for some months.

PRECAUTION

PmT-contaminated material should be soaked in 5% hypochlorite!
Wear disposable gloves during all the procedures!

Materials

DMEM (Gibco Life Technology LTD, Cat. No. 41965-039)

FBS (HyClone Laboratories, Cat. No. SH 30070.03)

Trypsin/EDTA solution (Gibco Life Technology LTD, Cat. No.25300-054)

Polybrene (Hexadimethrine bromide, Sigma Chemical **Co,** Cat. No. H9268).