

Name of cell lines: Balb/MK153

Culture medium: MCDB153 supplemented with

0.1 mM CaCl₂

5% dialyzed FBS¹

5 ng/ml EGF

10 µg/ml Gentamicin²

¹dialyzed against PBS(-) to reduce Ca⁺⁺ concentration

² can be changed to other antibiotics

Culture conditions: 37 °C with 5 % CO₂

Fluid renewal: every second day

Subculture interval: once a week

Subcultivation ratio: 1: 5

Subcultivation procedure:

remove medium, rinse cells twice with 0.02% EDTA/PBS, add 0.01~0.25% trypsin in 0.02% EDTA-PBS, incubate at 37 °C for 5-10 min, add fresh culture medium, centrifuge at 1200 rpm for 5 min, resuspend cells in fresh culture medium and dispense into new culture vessels.

Freeze medium: culture medium + 10% DMSO