

Revised by Christina McGee 4-5-2012

Cell Maintenance Media with Selection Agents (Zeocin, Blasticidin, Geneticin)

F12 from Gibco, 10% FBS, 1% Penicillin/Streptomycin, 1/10,000 Blasticidin, 1/4,000 Zeocin, 1/500 Geneticin

Blasticidin = 10mg/ml stock (pcDNA6)

Zeocin = 100mg/ml stock (pcDNA4)

Geneticin, G418, stock (pcDNA3, pIRES)

1. Obtain a 50mL conical tube and label as:
  - “Ham’s F-12 Media + 10% FBS + 1% Pen/Strep + 1ug/ml blast + 25ug/ml zeo + 100ug/ul gen”
  - Include date made and initials
2. Add 44.5ml of Ham’s F-12 Media to the labeled 50mL conical tube
3. Add 5ml of FBS
4. Add 500µl of Pen Strep
5. Add 5µl of blasticidin (Final Concentration (1µg/ml))
6. Add 12.5µl of zeocin (Final Concentration (25µg/ml))
7. Add 100µl of geneticin (Final Concentration (100µg/ml))
8. Mix by pipetting
9. Close the conical tube tightly and store in 4°C

To make selection media for establishing new cell lines, use 10x more selection agent for final concentrations of:

Add 50µl of blasticidin to 50ml of Ham’s F-12 Media + 10% FBS + 1% Pen/Strep media (Final Concentration (10µg/ml))

Add 125µl of zeocin to 50ml of Ham’s F-12 Media + 10% FBS + 1% Pen/Strep media (Final Concentration (250µg/ml))

Add 1000µl of geneticin to 50ml of Ham’s F-12 Media + 10% FBS + 1% Pen/Strep media (Final Concentration (1000µg/ml))