

Solutions for Primary Neuronal Culture

SEK 10/17/95

Dissociation Media (DM) - use within 1 month

Stock	100 ml	250 ml	500 ml
1 M Na ₂ SO ₄	8.18 ml	20.45 ml	40.9 ml
0.5 M K ₂ SO ₄	6 ml	15 ml	30 ml
1 M Mg Cl ₂	580 ul	1.45 ml	2.9 ml
100 mM CaCl ₂	252 ul	630 ul	1.26 ml
1 M Hepes	100 ul	250 ul	500 ul
30% Glucose	1.2 ml	3 ml	6 ml
0.5% Phenol Red	200 ul	500 ul	1 ml
1 N NaOH	~20 ul	~50 ul	~100 ul

Filter sterilize

OptiMem/20 mM glucose Optimem 500 ml 2.5 M glucose (store at 4°C) 4 ml BME growth media - prepare fresh.

Stock 100 ml 50 ml BME media 91.1 ml 45.6 ml bovine calf serum 5 ml 2.5 ml 1-1/2 2.4 ml 1.2 ml SVM 450 ul 225 ul ITS 50 ul 25 ul putrescine (8 ug/ml) 500 ul 250 ul transferrin (50 ug/ml) 500 ul 250 ul progesterone (100 uM) 12 ul 6 ul.

Filter sterilize.

DM/Ky. Mg 100 mls - prepare fresh.

DM 90 mls 10X Ky. Mg 10 ml enzyme solution - prepare fresh.

DM/Ky. Mg 9.7 ml cysteine-HCl 4.5 mg 1 N NaOH 25 ul papain latex 100 units.

Place at 37°C for ~10 min to dissolve papain.

pH with NaOH. F

Filter sterilize.

Place on ice until ~10 min prior to use.

Trypsin inhibitor - prepare fresh.

DM/Ky. Mg 9.7 ml trypsin inhibitor 100 mg mix/dissolve/adjust pH to 7.4 with ~40 ul 1 N NaOH.

Filter sterilize.

Place at 4°C until ~10 min prior to use.

Transferrin dissolve 100 mg in 20 ml 1X TC PBS.

Filter sterilize.

Store 1-1.5 ml aliquots at -20°C.