

Gastrointestinal Stem Cell Culture Protocol
 Modified KMS 06 May 2014
 HPW Lab

Supplies:

Matrigel (stock at -20)

Matrigel™ Basement Membrane Matrix (BD 354234) -20 10ml
 Thaw on ice and aliquot to single use sizes in eppendorf tubes store at -20

HBSS no Ca²⁺, no Mg²⁺, no phenol red (Life Technologies 14175-079) +4 1L

TrypLE™ Express (Life Technologies 12605-010) RT 100ml

EDTA (SIGMA E7889-100ml) RT 100ml

DMSO Hybri-Max (SIGMA D2650-100ml) RT 100ml

L-cell Medium (kept at +4C)

DMEM high glucose (SIGMA D6429-500ml) +4C 500ml

100x Penicillin/Streptomycin (SIGMA P4333-100ml) -20C 5ml

FBS (Heat inactivated) -20C 50ml

Primary Culture Medium (kept at +4C, make fresh weekly)

Advanced DEM/F12 (Invitrogen 12634-010) +4C 500ml

100x L-glutamine (SIGMA G7513-100ml) -20C 6.25ml

100x Penicillin/Streptomycin (SIGMA P4333-100ml) -20C 6.25ml

FBS (Heat inactivated) -20C 125ml

Washing Medium (kept at +4C, keep up to 1 month)

DMEM/F12 with HEPES (SIGMA D6421-500ml) +4C 500ml

100x L-glutamine (SIGMA G7513-100ml) -20C 5ml

100x Penicillin/Streptomycin (SIGMA P4333-100ml) -20C 5ml

FBS (Heat inactivated) -20C 50ml

Freezing Medium

Washing Medium +4C 8ml

FBS (Heat inactivated) -20C 1ml

DMSO (SIGMA D2650-100ml) RT 1ml

OR use:

Primary Culture Medium +4C 9ml

DMSO (SIGMA D2650-100ml) RT 1ml

Supplements

The CM contains unknown factors that can antagonize a factor of interest therefore, for short-term experiments (1-2 days) spheres can be formed by adding Wnt-3a (100ng/ml) and R-spondin 1(500ng/ml) in primary cell culture medium.

R-spondin 1 (Stock Solution: 250ng/ul)

Dissolve 50 ug R-spondin 1 (R&D 3474-RS-050) in 200ul PBS (+0.1%BSA)

Make aliquots (20-40ul) Keep at -80C

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Wnt-3a (Stock solution: 100ng/ul)
Dissolve 10 ug human Wnt-3a (R&D 5036-WN-010) in 100ul PBS (+0.1%BSA)
Make aliquots (10-20ul) Keep at -80C

Chemicals

G418 (SIGMA G8168-10ml)	50mg/ml
Hygromycin (InvivoGen ant-hg-1)	100mg/ml

ROCK Inhibitor

Addition of 10 uM Y-27632 improves cell viability by inhibiting anoikis.
Y-27632 (SIGMA Y0503-1mg) Stock 10mM in water
Dissolve 1mg in 312ul water to make 10mM stock of rock inhibitor, keep at -20C
Use at 1:1000 in 50% CM

TGFbeta1R Kinase inhibitor

Addition of 10 uM SB-431542 improves sphere formation by neutralizing TGFb from Matrigel
SB-431542 (EMD Millipore 616461-5mg) Stock 10mM in DMSO
Dissolve 5mg in 1.189ml DMSO to make 10mM stock of TGFb inhibitor, keep at -20C
Use at 1:1000 in 50% CM

(We don't currently use) **Collagenase solution**

Washing Medium	+4C	10ml
Collagenase type I (Invitrogen 17100-017)	+4C	20mg
Gentamicin (Invitrogen 15750060)	RT	10uL: final 50ug/ml