

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

Freeze Down/Thawing Organoids

Freezing Protocol

Supplies

Washing Medium

Freezing Medium (20% FBS, 10% DMSO)

Scratch and suspend matrigel by vigorous pipetting

Transfer suspension to 15ml tube with 5ml washing medium

Spin at 1000rpm for 5 min at +4C

Aspirate supernatant and suspend organoids gently in 500ul freezing medium per sample

Transfer to cryotube and freeze at -80C using appropriate freezing device

For long-term storage, transfer samples to liquid nitrogen

Thawing Protocol

Supplies

Washing Medium

Thaw Matrigel in ice water bath

Thaw Y-27632 and TGFbeta inhibitors (used at 1:1000)

Make sure have enough thawed 50% CM

Place cryotubes at 37C

Transfer organoids to 15 ml tube with 5ml washing medium

Spin at 300-1000rpm for 5 min at +4C to pellet organoids

Aspirate supernatant leaving ~200ul leftover, can pipet remove remaining volume

Suspend organoids in Matrigel (15-20ul per well)

Keep tubes on ice while suspend all samples for plating

Follow Plating Protocol in Crypt Culture Protocol