

# REGENERATIVE BIOLOGY

DR. JAMES THOMSON, DIRECTOR

## Thomson Lab Protocols

### Media and Reagents

Serum Free Media for human ES cells on MEFs: can last for 7-10 days

| Final Concentration          | Amount for 250ml Stock solution |
|------------------------------|---------------------------------|
| 80% DMEM-F12                 | 200ml                           |
| 20% KO Serum Replacer        | 50ml                            |
| 1% Non-essential Amino Acids | 2.5ml                           |
| 1mM L-glutamine (see recipe) | 2.5ml                           |
| 0.1mM b-mercaptoethanol      | part of L-glutamine             |
| 4ng/ml bFGF (see recipe)     | 0.5ml                           |

L-glutamine stock solution: Prepare on a per-use basis

- 0.146g L-glutamine
- 10ml Ca/Mg free PBS
- 7 ul b-mercaptoethanol

bFGF Stock: Store aliquots at -20 or -70

- 10ug vial of bFGF
- 5ml 0.1% Fraction V BSA
- Aliquot 0.5ml into sterile tubes

0.1% Fraction V BSA:

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- 100ml PBS with Ca and Mg
- Filter Sterilize

Collagenase IV Split Media: This media lasts 2-3 weeks

- Final concentration of 1mg/ml in DMEM-F12 media ie. 0.05g collagenase IV in 50ml DMEM-F12

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## Cell Freezing Media

### Cryopreservative Medium

| Final Concentration                | Amount for 10ml Stock Solution |
|------------------------------------|--------------------------------|
| 60% DMEM-F12                       | 6ml                            |
| 20% defined FBS                    | 2ml                            |
| 20% DMSO (do not filter sterilize) | 2ml                            |

### Resuspension Medium

| Final Concentration | Amount for 10ml Stock Solution |
|---------------------|--------------------------------|
| 80% DMEM-F12        | 8ml                            |
| 20% defined FBS     | 2ml                            |