Procedure to Prepare Media

# Materials Needed:

- \*\*Base Media:\*\* HEPES-buffered DMEM/F12 (Gibco, 11330032)

- \*\*Fetal Bovine Serum (FBS):\*\* Sigma-Aldrich, F4135

# Stock Solution Preparations:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Component | Initial Weight | Desired Stock Concentration | Solvent | Volume to Add |  |
| Gentamicin | 10.00 mg | 10 mg/mL | Water | 1.00 mL |  |
| Streptomycin | 10000.00 ug | 10000 ug/mL | Water | 1.00 mL |  |
| Noggin | 50.00 ug | 100 ug/mL | PBS with 0.1% BSA | 0.50 mL |  |
| Ascorbate-2-phosphate | 0.77 g | 200 mM | Water | 15.00 mL |  |
| Nicotinamide | 1.83 g | 1 M | Water | 15.00 mL |  |
| SB202190 | 5.00 mg | 10 mM | DMSO | 1.32 mL |  |
| A83-01 | 5.00 mg | 10 mM | DMSO | 10.67 mL |  |
| AlbuMAX | 1.50 g | 100 mg/mL | Water | 15.00 mL |  |
| KGF | 10.00 ug | 100 ug/mL | 0.1% BSA in PBS | 0.10 mL |  |
| R-spondin I | 100.00 ug | 100 ug/mL | PBS | 1.00 mL |  |
| EGF | 200.00 ug | 100 ug/mL | PBS | 2.00 mL |  |
| [Leu15]-Gastrin I | 0.94 mg | 1 mM | Water | 15.00 mL |  |
| Insulin | 100.00 mg | 10 mg/mL | 0.01 N HCl | 10.00 mL |  |
| Glucagon | 2.19 mg | 1 mM | Water | 15.00 mL |  |
| Wnt3a | 100.00 ug | 100 ug/mL | PBS | 1.00 mL |  |

Prepare the stock solutions as per the table above.

# Media Preparation Steps:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Step | Component | Desired Concentration | Stock Concentration | Volume to Add (μL) |
| 1 | HEPES-buffered DMEM/F12 | - | - | 12592.50 |
| 2 | Fetal Bovine Serum (FBS) | 10% v/v | - | 1500.00 |
| 3 | Gentamicin | 50.0 ug/mL | 10 mg/mL | 75.00 |
| 4 | Streptomycin | 100.0 ug/mL | 10000 ug/mL | 150.00 |
| 5 | Insulin/Transferrin/Selenium (ITS) | 1:100 dilution | None None | 150.00 |
| 6 | AlbuMAX | 1.0 mg/mL | 100 mg/mL | 150.00 |
| 7 | Ascorbate-2-phosphate | 200.0 uM | 200 mM | 15.00 |
| 8 | Nicotinamide | 10.0 mM | 1 M | 150.00 |
| 9 | SB202190 | 10.0 uM | 10 mM | 15.00 |
| 10 | KGF | 50.0 ng/mL | 100 ug/mL | 7.50 |
| 11 | EGF | 50.0 ng/mL | 100 ug/mL | 7.50 |
| 12 | Noggin | 100.0 ng/mL | 100 ug/mL | 15.00 |
| 13 | R-spondin I | 1.0 ug/mL | 100 ug/mL | 150.00 |
| 14 | A83-01 | 500.0 nM | 1.5000000000000002 mM | 5.00 |
| 15 | [Leu15]-Gastrin I | 10.0 nM | 0.029999999999999995 mM | 5.00 |
| 16 | Glucagon | 14.0 nM | 0.042 mM | 5.00 |
| 17 | Wnt3a | 50.0 ng/mL | 100 ug/mL | 7.50 |

\*\*Final Steps:\*\*

- Gently mix all components to ensure thorough mixing.

- Avoid creating bubbles.

- Use the media immediately or store at 4°C for up to one week.

- Protect from light if light-sensitive components are included.

# Appendix: Detailed Calculations

## Gentamicin

**Stock Solution Preparation:**- Initial Weight: 10.00 mg  
- Solvent: Water  
- Desired Stock Concentration: 10.00 mg/mL  
- Volume to Add: 1.00 mL  
**Calculations:**Volume (mL) = Mass (mg) / Concentration (mg/mL)  
Volume (mL) = 10.00 mg / 10.00 mg/mL = 1.00 mL  
**Media Preparation:**- Volume to Add: 75.00 μL  
**Calculations:**Volume (μL) = (Desired Conc × Final Volume (mL) × 1000) / Stock Conc  
Volume (μL) = (50.0 ug/mL × 15 mL × 1000) / 10 mg/mL  
Volume (μL) = (5.000000e+01 × 15 × 1000) / 1.000000e+04  
Volume (μL) = 75.00 μL

## Streptomycin

**Stock Solution Preparation:**- Initial Weight: 10000.00 ug  
- Solvent: Water  
- Desired Stock Concentration: 10000.00 ug/mL  
- Volume to Add: 1.00 mL  
**Calculations:**Volume (mL) = Mass (mg) / Concentration (mg/mL)  
Volume (mL) = 10.00 mg / 10.00 mg/mL = 1.00 mL  
**Media Preparation:**- Volume to Add: 150.00 μL  
**Calculations:**Volume (μL) = (Desired Conc × Final Volume (mL) × 1000) / Stock Conc  
Volume (μL) = (100.0 ug/mL × 15 mL × 1000) / 10000 ug/mL  
Volume (μL) = (1.000000e+02 × 15 × 1000) / 1.000000e+04  
Volume (μL) = 150.00 μL

## Noggin

**Stock Solution Preparation:**- Initial Weight: 50.00 ug  
- Solvent: PBS with 0.1% BSA  
- Desired Stock Concentration: 100.00 ug/mL  
- Volume to Add: 0.50 mL  
**Calculations:**Volume (mL) = Mass (mg) / Concentration (mg/mL)  
Volume (mL) = 0.05 mg / 0.10 mg/mL = 0.50 mL  
**Media Preparation:**- Volume to Add: 15.00 μL  
**Calculations:**Volume (μL) = (Desired Conc × Final Volume (mL) × 1000) / Stock Conc  
Volume (μL) = (100.0 ng/mL × 15 mL × 1000) / 100 ug/mL  
Volume (μL) = (1.000000e-01 × 15 × 1000) / 1.000000e+02  
Volume (μL) = 15.00 μL

## Ascorbate-2-phosphate

**Stock Solution Preparation:**- Initial Weight: 0.77 g  
- Solvent: Water  
- Desired Stock Concentration: 200.00 mM  
- Volume to Add: 15.00 mL  
**Calculations:**Moles = Mass (g) / Molecular Weight (g/mol)  
Moles = 0.774300 g / 258.10 g/mol = 0.003000 mol  
Volume (L) = Moles / Concentration (M)  
Volume (L) = 0.003000 mol / 2.000000e-01 M = 0.015000 L  
Volume (mL) = 0.015000 L \* 1000 = 15.00 mL  
**Media Preparation:**- Volume to Add: 15.00 μL  
**Calculations:**Volume (μL) = (Desired Conc × Final Volume (mL) × 1000) / Stock Conc  
Volume (μL) = (200.0 uM × 15 mL × 1000) / 200 mM  
Volume (μL) = (2.000000e-04 × 15 × 1000) / 2.000000e-01  
Volume (μL) = 15.00 μL

## Nicotinamide

**Stock Solution Preparation:**- Initial Weight: 1.83 g  
- Solvent: Water  
- Desired Stock Concentration: 1.00 M  
- Volume to Add: 15.00 mL  
**Calculations:**Moles = Mass (g) / Molecular Weight (g/mol)  
Moles = 1.831800 g / 122.12 g/mol = 0.015000 mol  
Volume (L) = Moles / Concentration (M)  
Volume (L) = 0.015000 mol / 1.000000e+00 M = 0.015000 L  
Volume (mL) = 0.015000 L \* 1000 = 15.00 mL  
**Media Preparation:**- Volume to Add: 150.00 μL  
**Calculations:**Volume (μL) = (Desired Conc × Final Volume (mL) × 1000) / Stock Conc  
Volume (μL) = (10.0 mM × 15 mL × 1000) / 1 M  
Volume (μL) = (1.000000e-02 × 15 × 1000) / 1.000000e+00  
Volume (μL) = 150.00 μL

## SB202190

**Stock Solution Preparation:**- Initial Weight: 5.00 mg  
- Solvent: DMSO  
- Desired Stock Concentration: 10.00 mM  
- Volume to Add: 1.32 mL  
**Calculations:**Moles = Mass (g) / Molecular Weight (g/mol)  
Moles = 0.005000 g / 377.40 g/mol = 0.000013 mol  
Volume (L) = Moles / Concentration (M)  
Volume (L) = 0.000013 mol / 1.000000e-02 M = 0.001325 L  
Volume (mL) = 0.001325 L \* 1000 = 1.32 mL  
**Media Preparation:**- Volume to Add: 15.00 μL  
**Calculations:**Volume (μL) = (Desired Conc × Final Volume (mL) × 1000) / Stock Conc  
Volume (μL) = (10.0 uM × 15 mL × 1000) / 10 mM  
Volume (μL) = (1.000000e-05 × 15 × 1000) / 1.000000e-02  
Volume (μL) = 15.00 μL

## A83-01

**Stock Solution Preparation:**- Initial Weight: 5.00 mg  
- Solvent: DMSO  
- Desired Stock Concentration: 1.50 mM  
- Volume to Add: 10.67 mL  
**Calculations:**Moles = Mass (g) / Molecular Weight (g/mol)  
Moles = 0.005000 g / 312.30 g/mol = 0.000016 mol  
Volume (L) = Moles / Concentration (M)  
Volume (L) = 0.000016 mol / 1.500000e-03 M = 0.010673 L  
Volume (mL) = 0.010673 L \* 1000 = 10.67 mL  
**Media Preparation:**- Volume to Add: 5.00 μL  
**Calculations:**Volume (μL) = (Desired Conc × Final Volume (mL) × 1000) / Stock Conc  
Volume (μL) = (500.0 nM × 15 mL × 1000) / 1.5000000000000002 mM  
Volume (μL) = (5.000000e-07 × 15 × 1000) / 1.500000e-03  
Volume (μL) = 5.00 μL

## AlbuMAX

**Stock Solution Preparation:**- Initial Weight: 1.50 g  
- Solvent: Water  
- Desired Stock Concentration: 100.00 mg/mL  
- Volume to Add: 15.00 mL  
**Calculations:**Volume (mL) = Mass (mg) / Concentration (mg/mL)  
Volume (mL) = 1500.00 mg / 100.00 mg/mL = 15.00 mL  
**Media Preparation:**- Volume to Add: 150.00 μL  
**Calculations:**Volume (μL) = (Desired Conc × Final Volume (mL) × 1000) / Stock Conc  
Volume (μL) = (1.0 mg/mL × 15 mL × 1000) / 100 mg/mL  
Volume (μL) = (1.000000e+03 × 15 × 1000) / 1.000000e+05  
Volume (μL) = 150.00 μL

## KGF

**Stock Solution Preparation:**- Initial Weight: 10.00 ug  
- Solvent: 0.1% BSA in PBS  
- Desired Stock Concentration: 100.00 ug/mL  
- Volume to Add: 0.10 mL  
**Calculations:**Volume (mL) = Mass (mg) / Concentration (mg/mL)  
Volume (mL) = 0.01 mg / 0.10 mg/mL = 0.10 mL  
**Media Preparation:**- Volume to Add: 7.50 μL  
**Calculations:**Volume (μL) = (Desired Conc × Final Volume (mL) × 1000) / Stock Conc  
Volume (μL) = (50.0 ng/mL × 15 mL × 1000) / 100 ug/mL  
Volume (μL) = (5.000000e-02 × 15 × 1000) / 1.000000e+02  
Volume (μL) = 7.50 μL

## R-spondin I

**Stock Solution Preparation:**- Initial Weight: 100.00 ug  
- Solvent: PBS  
- Desired Stock Concentration: 100.00 ug/mL  
- Volume to Add: 1.00 mL  
**Calculations:**Volume (mL) = Mass (mg) / Concentration (mg/mL)  
Volume (mL) = 0.10 mg / 0.10 mg/mL = 1.00 mL  
**Media Preparation:**- Volume to Add: 150.00 μL  
**Calculations:**Volume (μL) = (Desired Conc × Final Volume (mL) × 1000) / Stock Conc  
Volume (μL) = (1.0 ug/mL × 15 mL × 1000) / 100 ug/mL  
Volume (μL) = (1.000000e+00 × 15 × 1000) / 1.000000e+02  
Volume (μL) = 150.00 μL

## EGF

**Stock Solution Preparation:**- Initial Weight: 200.00 ug  
- Solvent: PBS  
- Desired Stock Concentration: 100.00 ug/mL  
- Volume to Add: 2.00 mL  
**Calculations:**Volume (mL) = Mass (mg) / Concentration (mg/mL)  
Volume (mL) = 0.20 mg / 0.10 mg/mL = 2.00 mL  
**Media Preparation:**- Volume to Add: 7.50 μL  
**Calculations:**Volume (μL) = (Desired Conc × Final Volume (mL) × 1000) / Stock Conc  
Volume (μL) = (50.0 ng/mL × 15 mL × 1000) / 100 ug/mL  
Volume (μL) = (5.000000e-02 × 15 × 1000) / 1.000000e+02  
Volume (μL) = 7.50 μL

## [Leu15]-Gastrin I

**Stock Solution Preparation:**- Initial Weight: 0.94 mg  
- Solvent: Water  
- Desired Stock Concentration: 0.03 mM  
- Volume to Add: 15.00 mL  
**Calculations:**Moles = Mass (g) / Molecular Weight (g/mol)  
Moles = 0.000942 g / 2094.40 g/mol = 0.000000 mol  
Volume (L) = Moles / Concentration (M)  
Volume (L) = 0.000000 mol / 3.000000e-05 M = 0.015000 L  
Volume (mL) = 0.015000 L \* 1000 = 15.00 mL  
**Media Preparation:**- Volume to Add: 5.00 μL  
**Calculations:**Volume (μL) = (Desired Conc × Final Volume (mL) × 1000) / Stock Conc  
Volume (μL) = (10.0 nM × 15 mL × 1000) / 0.029999999999999995 mM  
Volume (μL) = (1.000000e-08 × 15 × 1000) / 3.000000e-05  
Volume (μL) = 5.00 μL

## Insulin

**Stock Solution Preparation:**- Initial Weight: 100.00 mg  
- Solvent: 0.01 N HCl  
- Desired Stock Concentration: 10.00 mg/mL  
- Volume to Add: 10.00 mL  
**Calculations:**Volume (mL) = Mass (mg) / Concentration (mg/mL)  
Volume (mL) = 100.00 mg / 10.00 mg/mL = 10.00 mL

## Glucagon

**Stock Solution Preparation:**- Initial Weight: 2.19 mg  
- Solvent: Water  
- Desired Stock Concentration: 0.04 mM  
- Volume to Add: 15.00 mL  
**Calculations:**Moles = Mass (g) / Molecular Weight (g/mol)  
Moles = 0.002195 g / 3483.70 g/mol = 0.000001 mol  
Volume (L) = Moles / Concentration (M)  
Volume (L) = 0.000001 mol / 4.200000e-05 M = 0.015000 L  
Volume (mL) = 0.015000 L \* 1000 = 15.00 mL  
**Media Preparation:**- Volume to Add: 5.00 μL  
**Calculations:**Volume (μL) = (Desired Conc × Final Volume (mL) × 1000) / Stock Conc  
Volume (μL) = (14.0 nM × 15 mL × 1000) / 0.042 mM  
Volume (μL) = (1.400000e-08 × 15 × 1000) / 4.200000e-05  
Volume (μL) = 5.00 μL

## Wnt3a

**Stock Solution Preparation:**- Initial Weight: 100.00 ug  
- Solvent: PBS  
- Desired Stock Concentration: 100.00 ug/mL  
- Volume to Add: 1.00 mL  
**Calculations:**Volume (mL) = Mass (mg) / Concentration (mg/mL)  
Volume (mL) = 0.10 mg / 0.10 mg/mL = 1.00 mL  
**Media Preparation:**- Volume to Add: 7.50 μL  
**Calculations:**Volume (μL) = (Desired Conc × Final Volume (mL) × 1000) / Stock Conc  
Volume (μL) = (50.0 ng/mL × 15 mL × 1000) / 100 ug/mL  
Volume (μL) = (5.000000e-02 × 15 × 1000) / 1.000000e+02  
Volume (μL) = 7.50 μL