Procedure to Prepare Media

# Materials Needed:

- \*\*Base Media:\*\* HEPES-buffered DMEM/F12

- \*\*Serum:\*\* Fetal Bovine Serum (FBS)

# Stock Solution Preparations:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Component | Initial Weight | Stock Concentration | Solvent | Volume to Add | Cost per mL |
| Gentamicin | 10.00 mg | 10.00 mg/mL | Water | 1.00 mL | $44.25/mL |
| Streptomycin | 10000.00 ug | 10000.00 ug/mL | Water | 1.00 mL | $29.56/mL |
| Noggin | 50.00 ug | 100.00 ug/mL | PBS with 0.1% BSA | 0.50 mL | $404.26/mL |
| Ascorbate-2-phosphate | 0.77 g | 200.00 mM | Water | 15.00 mL | $6.31/mL |
| Nicotinamide | 1.83 g | 1.00 M | Water | 15.00 mL | $7.77/mL |
| SB202190 | 5.00 mg | 10.00 mM | DMSO | 1.32 mL | $104.16/mL |
| A83-01 | 5.00 mg | 1.50 mM | DMSO | 10.67 mL | $9.00/mL |
| AlbuMAX | 1.50 g | 100.00 mg/mL | Water | 15.00 mL | $9.20/mL |
| KGF | 10.00 ug | 100.00 ug/mL | 0.1% BSA in PBS | 0.10 mL | $1950.00/mL |
| R-spondin I | 100.00 ug | 100.00 ug/mL | PBS | 1.00 mL | $953.00/mL |
| EGF | 200.00 ug | 100.00 ug/mL | PBS | 2.00 mL | $73.50/mL |
| [Leu15]-Gastrin I | 0.94 mg | 0.03 mM | Water | 15.00 mL | $28.27/mL |
| Insulin | 100.00 mg | 10.00 mg/mL | 0.01 N HCl | 10.00 mL | $23.10/mL |
| Glucagon | 5.00 mg | 1.00 mM | Water | 1.44 mL | $203.70/mL |
| Wnt3a | 100.00 ug | 100.00 ug/mL | PBS | 1.00 mL | $434.00/mL |

Prepare the stock solutions as per the table above.

# Media Preparation Steps:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Step | Component | Desired Concentration | Volume to Add (μL) | Cost |
| 1 | HEPES-buffered DMEM/F12 | - | 12597.50 | - |
| 2 | Fetal Bovine Serum (FBS) | 10.0% v/v | 1500.00 | - |
| 3 | Gentamicin | 50.0 ug/mL | 75.00 | $3.32 |
| 4 | Streptomycin | 100.0 ug/mL | 150.00 | $4.43 |
| 5 | Noggin | 100.0 ng/mL | 15.00 | $6.06 |
| 6 | KGF | 50.0 ng/mL | 7.50 | $14.63 |
| 7 | EGF | 50.0 ng/mL | 7.50 | $0.55 |
| 8 | R-spondin I | 500.0 ng/mL | 75.00 | $71.48 |
| 9 | A83-01 | 500.0 nM | 5.00 | $0.30 |
| 10 | SB202190 | 10.0 uM | 15.00 | $1.56 |
| 11 | [Leu15]-Gastrin I | 10.0 nM | 5.00 | $4.44 |
| 12 | Glucagon | 1.0 uM | 15.00 | $3.06 |
| 13 | Ascorbate-2-phosphate | 1.0 mM | 75.00 | $0.47 |
| 14 | Nicotinamide | 10.0 mM | 150.00 | $1.17 |
| 15 | Insulin/Transferrin/Selenium (ITS) | 1:100 dilution | 150.00 | N/A |
| 16 | AlbuMAX | 1:100 dilution | 150.00 | $1.38 |
| 17 | Wnt3a | 50.0 ng/mL | 7.50 | $3.26 |

\*\*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.

\*\*Total Cost:\*\*

- Total Cost of Media: $116.10

- Cost per mL of Media: $7.74/mL