1. 1x Phosphate Buffer Saline, 10 mM (PBS)

<table>
<thead>
<tr>
<th>For 1000 mL</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>8.0 g</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.2 g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.2 g</td>
</tr>
<tr>
<td>Na₂HPO₄,2H₂O</td>
<td>1.15 g</td>
</tr>
<tr>
<td>Water</td>
<td>800 mL</td>
</tr>
</tbody>
</table>

Salts were dissolved and the pH was adjusted to 7.3 ± 0.1. Volume was made-up to 1 L using Milli Q water. Aliquoted into working volumes and autoclaved. Stored at room temperature.

2. MTT [3-(4, 5-dimethylthiaiazol-2)-2, 5-diphenyltetrazolium Bromide] Reagent (0.5%)

<table>
<thead>
<tr>
<th>For 10 mL</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>MTT</td>
<td>50 mg</td>
</tr>
<tr>
<td>PBS</td>
<td>10 mL</td>
</tr>
</tbody>
</table>

Dissolved and filter sterilized. Stored in amber reagent bottle at 2 – 8°C.

3. Sodium Dodecyl Sulphate [SDS] (25% w/v) solution

<table>
<thead>
<tr>
<th>For 100 mL</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS</td>
<td>25 g</td>
</tr>
</tbody>
</table>

Dissolved in warm WFI and pH was adjusted to 2.65 with 1M HCl. Volume was made-up to 100 mL using WFI. Filtered through Whatman No. 1 and stored at room temperature.
4. 0.25% Trypsin-EDTA solution

<table>
<thead>
<tr>
<th></th>
<th>For 100 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypsin</td>
<td>250 mg</td>
</tr>
<tr>
<td>EDTA</td>
<td>20 mg</td>
</tr>
<tr>
<td>PBS</td>
<td>100 mL</td>
</tr>
</tbody>
</table>

EDTA was dissolved in autoclaved PBS and pH was adjusted to 7.3 ± 0.1. Later 250 mg trypsin was dissolved in the solution. Reagent was filter sterilized and stored in small aliquots at –20°C. Working stock was stored at 2 – 8°C.

5. Freezing Media for r-CHO and TF-1 cells (FM)

<table>
<thead>
<tr>
<th></th>
<th>For 100 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>90 mL</td>
</tr>
<tr>
<td>DMSO</td>
<td>10 mL</td>
</tr>
</tbody>
</table>

Freshly prepared prior to use.

6. Growth Media for Control r-CHO cells (CGM)

<table>
<thead>
<tr>
<th></th>
<th>For 1000 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMEM</td>
<td>899 mL</td>
</tr>
<tr>
<td>FBS</td>
<td>100 mL</td>
</tr>
<tr>
<td>Gentamicin (80 mg/2 mL)</td>
<td>1 mL</td>
</tr>
</tbody>
</table>

Mixed and filter sterilized. Stored at 2 – 8°C.

7. Growth Media for partially adapted Test r-CHO cells (PGM)

<table>
<thead>
<tr>
<th></th>
<th>For 1000 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMEM</td>
<td>899 mL</td>
</tr>
<tr>
<td>FBS</td>
<td>1.5 mL</td>
</tr>
<tr>
<td>Milk</td>
<td>98.5 mL</td>
</tr>
<tr>
<td>Gentamicin (80 mg/2 mL)</td>
<td>1 mL</td>
</tr>
</tbody>
</table>

Mixed and filter sterilized. Stored at 2 – 8°C.
8. Growth Media for Test r-CHO cells (TGM)

<table>
<thead>
<tr>
<th>For 1000 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMEM (pH, 7.2)</td>
</tr>
<tr>
<td>Milk</td>
</tr>
<tr>
<td>Gentamycin (80mg/2mL)</td>
</tr>
<tr>
<td>Mixed and filter sterilized. Stored at 2 – 8°C.</td>
</tr>
</tbody>
</table>

9. Production Media for Control and Test r-CHO cells (PM)

<table>
<thead>
<tr>
<th>For 1000 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMEM/F12</td>
</tr>
<tr>
<td>NEAA’s (100X)</td>
</tr>
<tr>
<td>Insulin (35 mg/10 mL)</td>
</tr>
<tr>
<td>D-Glucose</td>
</tr>
<tr>
<td>L-Glutamine (100 mM)</td>
</tr>
<tr>
<td>Gentamycin (80 mg/2 mL)</td>
</tr>
<tr>
<td>Mixed, filter sterilized and stored in glass reagent bottle at 2 – 8°C.</td>
</tr>
</tbody>
</table>

10. Growth Media for TF-1 cells (GM)

<table>
<thead>
<tr>
<th>For 1000 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPMI-1640</td>
</tr>
<tr>
<td>FBS</td>
</tr>
<tr>
<td>Penicillin – Streptomyacin Solution [10,000 units Penicillin / mL with 10,000μg Streptomyacin / mL in 0.85% NaCl]</td>
</tr>
<tr>
<td>Sodium Pyruvate (100 μM)</td>
</tr>
<tr>
<td>GM-CSF (1μg/mL)</td>
</tr>
<tr>
<td>L-Glutamine (100 μM)</td>
</tr>
<tr>
<td>Mixed, filter sterilized and stored in glass bottle at 2 – 8°C.</td>
</tr>
</tbody>
</table>

11. Assay Media for TF-1 cells (AM)

<table>
<thead>
<tr>
<th>For 1000 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPMI-1640</td>
</tr>
<tr>
<td>FBS</td>
</tr>
<tr>
<td>Penicillin – Streptomyacin Solution [10,000 units Penicillin / mL with 10,000 μg Streptomyacin / mL in 0.85% NaCl]</td>
</tr>
<tr>
<td>Sodium Pyruvate (100 mM)</td>
</tr>
<tr>
<td>L-Glutamine (100 mM)</td>
</tr>
<tr>
<td>Mixed, filter sterilized and stored in glass reagent bottle at 2 – 8°C.</td>
</tr>
</tbody>
</table>
12. RP-HPLC Column details

<table>
<thead>
<tr>
<th>Main Column details</th>
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<tbody>
<tr>
<td><strong>Brand</strong></td>
</tr>
<tr>
<td><strong>Cat #</strong></td>
</tr>
<tr>
<td><strong>Type</strong></td>
</tr>
<tr>
<td><strong>Dia</strong></td>
</tr>
<tr>
<td><strong>Length</strong></td>
</tr>
<tr>
<td><strong>Porosity</strong></td>
</tr>
<tr>
<td><strong>Particle Size</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Guard Column details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brand</strong></td>
</tr>
<tr>
<td><strong>Cat #</strong></td>
</tr>
<tr>
<td><strong>Type</strong></td>
</tr>
<tr>
<td><strong>Dia</strong></td>
</tr>
<tr>
<td><strong>Length</strong></td>
</tr>
<tr>
<td><strong>Porosity</strong></td>
</tr>
<tr>
<td><strong>Particle Size</strong></td>
</tr>
</tbody>
</table>

13. RP-HPLC Buffers

**Mobile Phase A**

0.1% v/v Trifluoroacetic acid (TFA) in Water

1 mL of TFA was dissolved in 999 mL of water and filtered through 0.2 μ filter

**Mobile Phase B**

0.1% v/v Trifluoroacetic acid in Acetonitrile (ACN)

1 mL of TFA was dissolved in 999 mL of ACN and filtered through 0.2 μ filter

14. Acrylamide Solution: 30%

<table>
<thead>
<tr>
<th>For 100 mL of stock solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bis-acrylamide</td>
</tr>
<tr>
<td>Acrylamide</td>
</tr>
</tbody>
</table>

Dissolved in 40 mL of Milli-Q water and volume was made up to 100 mL. Filtered and stored at 2 – 8°C in a dark bottle.
15. Tris-HCl buffer (pH 6.8, 0.5 M)

<table>
<thead>
<tr>
<th></th>
<th>For 100 mL of stock solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris</td>
<td>6 g</td>
</tr>
<tr>
<td>Milli-Q Water</td>
<td>80.7 mL</td>
</tr>
</tbody>
</table>

pH was adjusted to 6.8 with 1M HCl and then volume was made up to 100 mL. Stored at 2 – 8°C.

16. Tris-HCl buffer (pH 8.8, 1.5 M)

<table>
<thead>
<tr>
<th></th>
<th>For 100 mL of stock solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris</td>
<td>18 g</td>
</tr>
<tr>
<td>Milli-Q Water</td>
<td>80 mL</td>
</tr>
</tbody>
</table>

pH was adjusted to 6.8 with 1M HCl and then volume was made up to 100 mL. Stored at 2 – 8°C.

17. Sodium dodecyl sulfate (20% w/v)

<table>
<thead>
<tr>
<th></th>
<th>For 100 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS</td>
<td>20 g</td>
</tr>
<tr>
<td>Milli-Q water</td>
<td>100 mL</td>
</tr>
</tbody>
</table>

SDS was dissolved in warm Milli-Q Water. Filtered through Whatman No. 1 filter paper and stored at room temperature.

18. Ammonium per sulfate (10% w/v)

<table>
<thead>
<tr>
<th></th>
<th>For 1 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>APS</td>
<td>100 mg</td>
</tr>
<tr>
<td>Milli-Q water</td>
<td>1 mL</td>
</tr>
</tbody>
</table>

APS was dissolved in Milli-Q Water and stored at room temperature.
19. Butylated water (Butanol saturated with water)

<table>
<thead>
<tr>
<th></th>
<th>For 100 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>50 mL</td>
</tr>
<tr>
<td>Butanol</td>
<td>50 mL</td>
</tr>
</tbody>
</table>

Mixture was stirred for 30 minutes on magnetic stirrer. Solvent layer was allowed to separate and upper layer was used to overlay the gel.

20. SDS- gel: Tris–glycine running buffer

<table>
<thead>
<tr>
<th></th>
<th>For 1000 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris base</td>
<td>3.03 g</td>
</tr>
<tr>
<td>Glycine</td>
<td>14.4 g</td>
</tr>
<tr>
<td>SDS</td>
<td>1.0 g</td>
</tr>
</tbody>
</table>

All the contents were dissolved in 500 mL WFI and volume was made up to 1 L. Stored at room temperature.

21. Non-reducing sample loading buffer

<table>
<thead>
<tr>
<th></th>
<th>For 20 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris</td>
<td>0.907 g</td>
</tr>
<tr>
<td>SDS</td>
<td>2.4 g</td>
</tr>
<tr>
<td>Bromophenol blue</td>
<td>24 mg</td>
</tr>
<tr>
<td>Glycerol</td>
<td>12 mL</td>
</tr>
</tbody>
</table>

Tris was dissolved in 10 mL of Milli Q water and the pH was adjusted to 6.8 with 1M HCl. SDS was added and kept at 37°C in a water bath until it got dissolved completely. Bromophenol blue & Glycerol were added later and kept at 37°C in a water bath. Finally volume was made up to 20 mL and stored at room temperature.
22. SDS Gel Staining Solution (Silver staining)

<table>
<thead>
<tr>
<th>Solutions</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixing solution</td>
<td>50 mL methanol, 10 mL acetic acid in 40 mL WFI</td>
</tr>
<tr>
<td>5% Methanol (v/v)</td>
<td>5 mL methanol in 95 mL WFI</td>
</tr>
<tr>
<td>Sensitizing Solution</td>
<td>50 mg sodium thiosulphate was dissolved in 250 mL WFI</td>
</tr>
<tr>
<td>Staining solution</td>
<td>500 mg silver nitrate was dissolved in 250 mL WFI.</td>
</tr>
<tr>
<td>Developing solution</td>
<td>Sodium carbonate (7 g) was dissolved in 200 mL WFI + 5 mL sodium thiosulphate and 125 µL of formaldehyde. Volume was made up to 250 mL with WFI.</td>
</tr>
<tr>
<td>Stopping Solution</td>
<td>Glacial acetic acid (25 mL) was added to 225 mL WFI</td>
</tr>
</tbody>
</table>

23. Growth media for test cells (for 1000 mL) with different pH

<table>
<thead>
<tr>
<th>S. No</th>
<th>DMEM, mL</th>
<th>Milk, mL</th>
<th>Gentamycin (80 mg/2 mL), mL</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>899</td>
<td>100</td>
<td>1</td>
<td>7.0</td>
</tr>
<tr>
<td>2</td>
<td>899</td>
<td>100</td>
<td>1</td>
<td>7.2</td>
</tr>
<tr>
<td>3</td>
<td>899</td>
<td>100</td>
<td>1</td>
<td>7.4</td>
</tr>
</tbody>
</table>
### Growth media with different serum, milk, insulin, NEAA, Glutamine, fibronectin concentrations (total volume, 1000 mL)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>DMEM, mL</th>
<th>FBS, mL</th>
<th>Milk, mL</th>
<th>Insulin (35mg/10mL), μL</th>
<th>NEAA (100 X), mL</th>
<th>L-Glutamine (100 mM), mL</th>
<th>Fibronectin, μg</th>
<th>Gentamycin (80 mg/2 mL), mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>899.00</td>
<td>1.5</td>
<td>98.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>899.00</td>
<td>1.0</td>
<td>99.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>375.0</td>
</tr>
<tr>
<td>3</td>
<td>892.85</td>
<td>1.0</td>
<td>99.0</td>
<td>150</td>
<td>-</td>
<td>6.0</td>
<td>375.0</td>
<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>895.85</td>
<td>1.0</td>
<td>99.0</td>
<td>-</td>
<td>3.0</td>
<td>-</td>
<td>375.0</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>898.85</td>
<td>0.5</td>
<td>99.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>375.0</td>
<td>1.0</td>
</tr>
<tr>
<td>6</td>
<td>892.85</td>
<td>0.5</td>
<td>99.5</td>
<td>150</td>
<td>-</td>
<td>6.0</td>
<td>375.0</td>
<td>1.0</td>
</tr>
<tr>
<td>7</td>
<td>895.85</td>
<td>0.5</td>
<td>99.5</td>
<td>150</td>
<td>3.0</td>
<td>-</td>
<td>375.0</td>
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<tr>
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<td>899.00</td>
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<td>-</td>
<td>-</td>
<td>375.0</td>
<td>1.0</td>
</tr>
<tr>
<td>9</td>
<td>892.85</td>
<td>-</td>
<td>100</td>
<td>150</td>
<td>-</td>
<td>6.0</td>
<td>375.0</td>
<td>1.0</td>
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<tr>
<td>10</td>
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<td>150</td>
<td>3.0</td>
<td>-</td>
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<tr>
<td>11</td>
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<td>-</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>187.5</td>
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<td>12</td>
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<td>-</td>
<td>100</td>
<td>150</td>
<td>-</td>
<td>6.0</td>
<td>187.5</td>
<td>1.0</td>
</tr>
<tr>
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<td>150</td>
<td>3.0</td>
<td>-</td>
<td>187.5</td>
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</tr>
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<td>-</td>
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<td>-</td>
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<td>75.00</td>
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<td>100</td>
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<td>1.0</td>
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<td>100</td>
<td>150</td>
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<td>6.0</td>
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<td>1.0</td>
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<td>100</td>
<td>150</td>
<td>3.0</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
</tr>
</tbody>
</table>
25. Production media (for 1000 mL) with different concentrations of supplements and pH

<table>
<thead>
<tr>
<th>S. No</th>
<th>DMEM/F12, mL</th>
<th>NEAA (100 X), mL</th>
<th>Insulin, (35 mg/mL) µL</th>
<th>D-Glucose, g</th>
<th>L-Glutamine (100 mM), mL</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>994.7</td>
<td>1</td>
<td>275</td>
<td>2.25</td>
<td>4</td>
<td>7.2</td>
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<tr>
<td>2</td>
<td>990.7</td>
<td>5</td>
<td>275</td>
<td>2.25</td>
<td>4</td>
<td>7.2</td>
</tr>
<tr>
<td>3</td>
<td>990.7</td>
<td>1</td>
<td>275</td>
<td>2.25</td>
<td>8</td>
<td>7.2</td>
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<tr>
<td>4</td>
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<td>6.75</td>
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<td>7.2</td>
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<td>2.25</td>
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<td>7.2</td>
</tr>
<tr>
<td>7</td>
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<td>2.25</td>
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<td>7.2</td>
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<tr>
<td>8</td>
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<td>7.2</td>
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<td>275</td>
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<td>7.2</td>
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<td>25</td>
<td>6.75</td>
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<td>7.2</td>
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<tr>
<td>14</td>
<td>986.7</td>
<td>5</td>
<td>275</td>
<td>6.75</td>
<td>8</td>
<td>7.2</td>
</tr>
<tr>
<td>15</td>
<td>990.9</td>
<td>3</td>
<td>150</td>
<td>4.50</td>
<td>6</td>
<td>7.2</td>
</tr>
<tr>
<td>16</td>
<td>987.0</td>
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<td>25</td>
<td>6.75</td>
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<tr>
<td>17</td>
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<tr>
<td>18</td>
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<tr>
<td>19</td>
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<td>6.75</td>
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<tr>
<td>20</td>
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<tr>
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<td>7.2</td>
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<tr>
<td>22</td>
<td>995.0</td>
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<td>25</td>
<td>6.75</td>
<td>4</td>
<td>7.2</td>
</tr>
<tr>
<td>23</td>
<td>986.7</td>
<td>5</td>
<td>275</td>
<td>6.75</td>
<td>8</td>
<td>7.2</td>
</tr>
<tr>
<td>24</td>
<td>990.9</td>
<td>3</td>
<td>150</td>
<td>4.50</td>
<td>6</td>
<td>7.2</td>
</tr>
<tr>
<td>25</td>
<td>990.9</td>
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<td>150</td>
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<tr>
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<td>4</td>
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<td>275</td>
<td>2.25</td>
<td>8</td>
<td>7.2</td>
</tr>
<tr>
<td>28</td>
<td>990.7</td>
<td>1</td>
<td>275</td>
<td>6.75</td>
<td>8</td>
<td>7.2</td>
</tr>
<tr>
<td>29</td>
<td>986.7</td>
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<td>275</td>
<td>2.25</td>
<td>8</td>
<td>7.2</td>
</tr>
<tr>
<td>30</td>
<td>987.0</td>
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<td>25</td>
<td>2.25</td>
<td>8</td>
<td>7.2</td>
</tr>
<tr>
<td>31</td>
<td>987.0</td>
<td>5</td>
<td>25</td>
<td>6.75</td>
<td>8</td>
<td>7.2</td>
</tr>
<tr>
<td>32</td>
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<td>25</td>
<td>2.25</td>
<td>4</td>
<td>7.2</td>
</tr>
<tr>
<td>33</td>
<td>991.0</td>
<td>5</td>
<td>25</td>
<td>6.75</td>
<td>4</td>
<td>7.2</td>
</tr>
<tr>
<td>34</td>
<td>994.7</td>
<td>1</td>
<td>275</td>
<td>2.25</td>
<td>4</td>
<td>7.2</td>
</tr>
<tr>
<td>35</td>
<td>994.7</td>
<td>1</td>
<td>275</td>
<td>6.75</td>
<td>4</td>
<td>7.2</td>
</tr>
<tr>
<td>36</td>
<td>991.0</td>
<td>1</td>
<td>25</td>
<td>2.25</td>
<td>8</td>
<td>7.2</td>
</tr>
<tr>
<td>37</td>
<td>990.9</td>
<td>3</td>
<td>150</td>
<td>4.50</td>
<td>6</td>
<td>7.2</td>
</tr>
<tr>
<td>38</td>
<td>991.0</td>
<td>1</td>
<td>25</td>
<td>6.75</td>
<td>8</td>
<td>7.2</td>
</tr>
<tr>
<td>39</td>
<td>990.7</td>
<td>3</td>
<td>150</td>
<td>4.50</td>
<td>6</td>
<td>7.0</td>
</tr>
<tr>
<td>40</td>
<td>990.7</td>
<td>3</td>
<td>150</td>
<td>4.50</td>
<td>6</td>
<td>7.2</td>
</tr>
<tr>
<td>41</td>
<td>990.7</td>
<td>3</td>
<td>150</td>
<td>4.50</td>
<td>6</td>
<td>7.4</td>
</tr>
</tbody>
</table>
26. HPLC column details used for Peptide Mapping

<table>
<thead>
<tr>
<th>Column</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand Name</td>
<td>ACE</td>
</tr>
<tr>
<td>Catalogue Number</td>
<td>ACE-221-2546</td>
</tr>
<tr>
<td>Type of Column</td>
<td>Reverse phase C18 column</td>
</tr>
<tr>
<td>Specification of Column</td>
<td>Diameter: 4.6mm, Length: 250 mm, Porosity: 300Å, Particle size: 5 Micron</td>
</tr>
</tbody>
</table>

| Guard column                  |       |
| Brand Name                    | Zorbex 300 SB (Agilent) |
| Catalogue Number              | 820950-921 |
| Type of Column                | Reverse phase Zorbax 300 SB-C18 |
| Specification of Column       | Diameter: 4.6mm, Length: 35 mm, Porosity: 300Å, Particle size: 5 Micron |

27. Tris Glycine Buffer, 10X

<table>
<thead>
<tr>
<th>For 100 mL</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>14.4 g</td>
</tr>
<tr>
<td>Tris</td>
<td>3.03 g</td>
</tr>
</tbody>
</table>

Dissolved in 80 mL of warm distilled water and volume was made up to 100 mL. Stored at room temperature. Used within 3 months from the date of preparation.

28. Tris- Glycine Buffer (1X) for Electro-Transfer

<table>
<thead>
<tr>
<th>For 100 mL</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris glycine buffer (10X)</td>
<td>10 mL</td>
</tr>
<tr>
<td>Methanol</td>
<td>10 mL</td>
</tr>
</tbody>
</table>

Dissolved and the volume was made up to 100 mL with WFI. Made fresh each time. The solution was chilled at -20°C for at least 1 h before use.
29. Tris-Acetate Buffer

For 1000 mL

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>0.294 g</td>
</tr>
<tr>
<td>Tris amino-methane (Tris-base)</td>
<td>12.11 g</td>
</tr>
</tbody>
</table>

Contents were dissolved in 800 mL of WFI, pH was adjusted to 8.5 by adding glacial acetic acid and volume was made up to 1 L with WFI. Stored at 2 - 8°C.

30. Trypsin solution

For 10 mL

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypsin</td>
<td>10 mg</td>
</tr>
</tbody>
</table>

Dissolved in 8 mL of sterile WFI and volume was made up to 10 mL. Stored at -20°C in aliquots of 200-500 µL in microtubes. Used one aliquot each time to avoid repeated freezing and thawing.

31. Tris-Buffered Saline (TBS), 10X

For 1000 mL

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris base</td>
<td>30 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>80 g</td>
</tr>
<tr>
<td>KCl</td>
<td>20 g</td>
</tr>
</tbody>
</table>

Dissolved in 800 mL of WFI. Adjusted the pH to 7.4 with 1N HCl and volume was made up to 1000 mL with WFI.

32. Tris-Buffered Saline (TBS) Buffer, 1X

For 100 mL

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X TBS</td>
<td>10 mL</td>
</tr>
<tr>
<td>WFI</td>
<td>100 mL</td>
</tr>
</tbody>
</table>

Mixed and stored at room temperature.
33. 0.01 M NaOH

<table>
<thead>
<tr>
<th>For 1000 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M NaOH</td>
</tr>
<tr>
<td>WFI</td>
</tr>
</tbody>
</table>

10 mL of 1 M NaOH was added to 990 mL of WFI and stored at room temperature.

34. 1 M NaOH+1 M NaCl

<table>
<thead>
<tr>
<th>For 1000 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
</tr>
<tr>
<td>1 M NaOH</td>
</tr>
</tbody>
</table>

NaCl was dissolved in 800 mL of 1 M NaOH and volume was made up to 1 L using WFI. Filtered through 0.22 μm filter and stored at room temperature.

35. Blocking Buffer

<table>
<thead>
<tr>
<th>For 100 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skimmed milk powder</td>
</tr>
<tr>
<td>1X TBS</td>
</tr>
</tbody>
</table>

Dissolved and volume was made up to 100 mL with 1X TBS. Stored at 2 - 8°C.
36. Sodium Azide Stock, 5%

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>For 100 mL</td>
<td></td>
</tr>
<tr>
<td>Sodium azide</td>
<td>5 g</td>
</tr>
<tr>
<td>WFI</td>
<td>100 mL</td>
</tr>
<tr>
<td>Stored at room temperature</td>
<td></td>
</tr>
</tbody>
</table>

37. Primary Antibody solution

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>For 25 mL</td>
<td></td>
</tr>
<tr>
<td>Rabbit anti-EPO antibody</td>
<td>25 μL</td>
</tr>
<tr>
<td>Blocking buffer</td>
<td>24.725 mL</td>
</tr>
<tr>
<td>5% Sodium azide</td>
<td>250 μL</td>
</tr>
<tr>
<td>Stored at 2 - 8°C</td>
<td></td>
</tr>
</tbody>
</table>

38. Secondary Antibody (probe) solution

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>For 25 mL</td>
<td></td>
</tr>
<tr>
<td>Goat anti-rabbit IgG conjugated to Alkaline phosphatase enzyme</td>
<td>12.5 μL</td>
</tr>
<tr>
<td>Blocking buffer</td>
<td>24.74 mL</td>
</tr>
<tr>
<td>5% Sodium azide</td>
<td>250 μL</td>
</tr>
<tr>
<td>Stored at 2 - 8°C</td>
<td></td>
</tr>
</tbody>
</table>

39. IEF Gel loading buffer, 2X

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>For 10 mL</td>
<td></td>
</tr>
<tr>
<td>Glycerol (60 %)</td>
<td>6 mL</td>
</tr>
<tr>
<td>Ampholyte (0.4 %)</td>
<td>400 μL</td>
</tr>
<tr>
<td>(pH Range- 2.5 to 5.5)</td>
<td></td>
</tr>
<tr>
<td>Mixed and volume was made to 10 mL with WFI. Stored at 2 - 8°C</td>
<td></td>
</tr>
</tbody>
</table>
40. 1M Ortho-Phosphoric Acid

<table>
<thead>
<tr>
<th></th>
<th>For 1000 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ortho-Phosphoric acid</td>
<td>57.3 mL</td>
</tr>
<tr>
<td>WFI</td>
<td>942.7 mL</td>
</tr>
</tbody>
</table>

Stored at room temperature.

41. Anode Buffer (Lower tank): 25 mM Ortho-Phosphoric Acid

<table>
<thead>
<tr>
<th></th>
<th>For 1000 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1M Ortho Phosphoric Acid</td>
<td>25 mL</td>
</tr>
<tr>
<td>WFI</td>
<td>975 mL</td>
</tr>
</tbody>
</table>

Stored at room temperature. Prepared fresh and chilled before use.

42. 1M NaOH

<table>
<thead>
<tr>
<th></th>
<th>For 1000 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOH</td>
<td>40 g</td>
</tr>
<tr>
<td>WFI</td>
<td>500 mL</td>
</tr>
</tbody>
</table>

Dissolved and volume was made up to 1000 mL with WFI. Stored at room temperature.

43. Cathode Buffer: 25 mM NaOH

<table>
<thead>
<tr>
<th></th>
<th>For 1000 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M NaOH</td>
<td>25 mL</td>
</tr>
<tr>
<td>WFI</td>
<td>975 mL</td>
</tr>
</tbody>
</table>

Stored at room temperature. Prepared fresh and chilled before use.
### 44. 10 % TCA

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>For 100 mL</td>
<td></td>
</tr>
<tr>
<td>TCA</td>
<td>10 g</td>
<td></td>
</tr>
<tr>
<td>WFI</td>
<td>100 mL</td>
<td></td>
</tr>
</tbody>
</table>

Dissolved and stored at room temperature. Prepared fresh and chilled before use.

### 45. 1 % TCA

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>For 1000 mL</td>
<td></td>
</tr>
<tr>
<td>10% TCA</td>
<td>100 mL</td>
<td></td>
</tr>
<tr>
<td>WFI</td>
<td>900 mL</td>
<td></td>
</tr>
</tbody>
</table>

Stored at room temperature.

### 46. Coomassie Brilliant Blue staining solution

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>For 200 mL</td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>100 mL</td>
<td></td>
</tr>
<tr>
<td>Glacial acetic acid</td>
<td>20 mL</td>
<td></td>
</tr>
<tr>
<td>Coomassie Brilliant Blue R-250</td>
<td>0.5 g</td>
<td></td>
</tr>
<tr>
<td>WFI</td>
<td>80 mL</td>
<td></td>
</tr>
</tbody>
</table>

Coomassie blue was dissolved in methanol and then acetic acid was added. Volume was made up to 200 mL with WFI and stored at room temperature.
47. SDS Gel De-staining Solution

<table>
<thead>
<tr>
<th>For 1000 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glacial acetic acid</td>
</tr>
<tr>
<td>Methanol</td>
</tr>
<tr>
<td>WFI</td>
</tr>
<tr>
<td>Stored at room temperature.</td>
</tr>
</tbody>
</table>

48. N-Acetyl Neuraminic Acid (Sialic Acid)

<table>
<thead>
<tr>
<th>For 10 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Acetyl Neuraminic Acid</td>
</tr>
<tr>
<td>WFI</td>
</tr>
<tr>
<td>Dissolved in 9 mL of WFI and volume was made up to 10 mL with WFI. Stored at 2 - 8°C</td>
</tr>
</tbody>
</table>

49. Resorcinol Stock solution

<table>
<thead>
<tr>
<th>For 50 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resorcinol</td>
</tr>
<tr>
<td>WFI</td>
</tr>
<tr>
<td>Resorcinol was dissolved in 45 mL of WFI and volume was made up to 50 mL. Stored at room temperature.</td>
</tr>
</tbody>
</table>

50. Copper Sulfate Solution

<table>
<thead>
<tr>
<th>For 1 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper sulfate</td>
</tr>
<tr>
<td>WFI</td>
</tr>
<tr>
<td>Copper sulfate was dissolved in 1 mL of WFI and stored at room temperature.</td>
</tr>
</tbody>
</table>
51. Resorcinol Reagent

<table>
<thead>
<tr>
<th>For 100 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. HCl</td>
</tr>
<tr>
<td>Resorcinol stock</td>
</tr>
<tr>
<td>Copper sulfate solution</td>
</tr>
<tr>
<td>WFI</td>
</tr>
</tbody>
</table>

Mixed all the contents well and stored at 2- 8°C.

52. Organic Solvent

<table>
<thead>
<tr>
<th>For 50 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butanol</td>
</tr>
<tr>
<td>Butyl acetate</td>
</tr>
</tbody>
</table>

Mixed and stored at room temperature.

53. Phosphate Buffer

<table>
<thead>
<tr>
<th>For 1000 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disodium hydrogen phosphate anhydrous</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
</tr>
<tr>
<td>Sodium chloride</td>
</tr>
</tbody>
</table>

Salts were dissolved in 800 mL of WFI and pH was adjusted to 7.2. Final volume was made up to 1L with WFI and stored at room temperature.

54. 0.1 M NaOH reagent

<table>
<thead>
<tr>
<th>For 1000 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M NaOH</td>
</tr>
<tr>
<td>WFI</td>
</tr>
</tbody>
</table>

100 mL of 1 M NaOH was added to 900 mL of WFI and stored at room temperature.
55. Loading Buffer used for Affinity Chromatography

<table>
<thead>
<tr>
<th>For 8 L</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>KH$_2$PO$_4$</td>
<td>1.6 g</td>
</tr>
<tr>
<td>Na$_2$PO$_4$</td>
<td>17.28 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>64.0 g</td>
</tr>
<tr>
<td>KCl</td>
<td>1.6 g</td>
</tr>
<tr>
<td>WFI</td>
<td>7.5 L</td>
</tr>
</tbody>
</table>

Salts were dissolved in 7.5 L WFI and pH was adjusted to 7.2 ± 0.2 using 6 M HCl. Volume was made to 8 L using WFI, filtered through 0.22 μm filter and stored at room temperature.

56. Elution Buffer used for Affinity Chromatography

<table>
<thead>
<tr>
<th>For 2.5 L</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>KH$_2$PO$_4$</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Na$_2$PO$_4$</td>
<td>5.4 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.515 g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.5 g</td>
</tr>
<tr>
<td>WFI</td>
<td>2.2 L</td>
</tr>
</tbody>
</table>

Salts were dissolved in 2.2 L WFI and pH was adjusted to 7.2 ± 0.2 using 6 M HCl / 1 M NaOH. Volume was made up to 2.5 L using WFI, filtered through 0.22 μm filter and stored at room temperature.

57. Sanitization Buffer used for Affinity Chromatography

<table>
<thead>
<tr>
<th>For 1.2 L</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>IPA (Iso-propyl alcohol)</td>
<td>360 mL</td>
</tr>
<tr>
<td>WFI</td>
<td>840 mL</td>
</tr>
</tbody>
</table>

Filtered through 0.22 μm filter and stored at room temperature.
### 58. Affinity Storage Buffer

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH₂PO₄</td>
<td>10.88 g</td>
</tr>
<tr>
<td>IPA</td>
<td>160 mL</td>
</tr>
<tr>
<td>WFI</td>
<td>500 mL</td>
</tr>
</tbody>
</table>

KH₂PO₄ was dissolved in 500 mL of WFI, pH was adjusted to 8.0 ± 0.2 using 2 M NaOH and then 160 mL of IPA was added. Volume made up to 800 mL using WFI and later filtered through 0.22 µm filter. Stored at room temperature.

### 59. Equilibration Buffer used for Diafiltration Or Cation Exchange (20 mM Tris)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris Base</td>
<td>1.935 g</td>
</tr>
<tr>
<td>WFI</td>
<td>750 mL</td>
</tr>
</tbody>
</table>

Tris base was dissolved in 750 mL WFI, pH was adjusted to 7.0 ± 0.2 using 6 M HCl. Volume was made up to 800 mL using WFI and filtered through 0.22 µm filter. Stored at room temperature.

### 60. Regeneration Buffer used for Cation Exchange

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>13.09 g</td>
</tr>
<tr>
<td>Tris</td>
<td>3.876 g</td>
</tr>
<tr>
<td>WFI</td>
<td>1400 mL</td>
</tr>
</tbody>
</table>

Ingredients were dissolved in 1400 mL WFI, pH was adjusted to 8.0 ± 0.2 using 6 M HCl and volume was made up to 1600 mL using WFI. Filtered through 0.22 µm filter and stored at room temperature.
61. 1 M Tris Base

<table>
<thead>
<tr>
<th></th>
<th>For 500 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris Base</td>
<td>60.57 g</td>
</tr>
<tr>
<td>WFI</td>
<td>300 mL</td>
</tr>
</tbody>
</table>

Tris base was dissolved in 300 mL WFI and volume was made up to 500 mL using WFI. Filtered through 0.22 μm filter and stored at room temperature.

62. Isopropyl alcohol (70%)

<table>
<thead>
<tr>
<th></th>
<th>For 100 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isopropyl alcohol</td>
<td>70 mL</td>
</tr>
<tr>
<td>Milli-Q</td>
<td>30 mL</td>
</tr>
</tbody>
</table>

Sterile filtered with 0.22μm membrane and stored at room temperature.

63. Loading Buffer used for Anion Exchange Chromatography-I

<table>
<thead>
<tr>
<th></th>
<th>For 2 L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris Base</td>
<td>4.85 g</td>
</tr>
<tr>
<td>WFI</td>
<td>1900 mL</td>
</tr>
</tbody>
</table>

Tris base was dissolved in 1900 mL WFI, pH was adjusted to 8 ± 0.2 using 6 M HCl. Volume was made up to 2.0 L. Filtered through 0.22 μm filter and stored at room temperature.
64. Elution Buffer used for Anion Exchange Chromatography-I

<table>
<thead>
<tr>
<th>For 1.5 L</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris Base</td>
<td>3.634 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>12.27 g</td>
</tr>
<tr>
<td>WFI</td>
<td>1.2 L</td>
</tr>
</tbody>
</table>

Ingredients were dissolved in 1.2 L of WFI, pH was adjusted to 8.0 ± 0.2 using 6 M HCl and volume was made up to 1.5 L using WFI. Filtered through 0.22 μm filter and stored at room temperature.

65. Loading Buffer used for Anion Exchange Chromatography-II

<table>
<thead>
<tr>
<th>For 600 mL</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris Base</td>
<td>3.634 g</td>
</tr>
<tr>
<td>WFI</td>
<td>500 mL</td>
</tr>
</tbody>
</table>

Tris base was dissolved in 500 mL WFI, pH was adjusted to 8 ± 0.2 with 6 M HCl and volume was made up to 600 mL using WFI. Filtered through 0.22 μm filter and stored at room temperature.

66. Elution Buffer used for Anion Exchange Chromatography-II

<table>
<thead>
<tr>
<th>For 400 mL</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris Base</td>
<td>2.423 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>2.3376 g</td>
</tr>
<tr>
<td>WFI</td>
<td>300 mL</td>
</tr>
</tbody>
</table>

Ingredients were dissolved in 300 mL of WFI, pH was adjusted to 8.0 ± 0.2 using 6 M HCl and volume was made up to 400 mL using WFI. Filtered through 0.22 μm filter and stored at room temperature.
### 67. 1 M Acetic Acid reagent

<table>
<thead>
<tr>
<th>For 200 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acetic acid</strong></td>
</tr>
<tr>
<td><strong>WFI</strong></td>
</tr>
</tbody>
</table>

Dissolved and filtered through 0.22 μm filter. Stored at room temperature.

### 68. Acetate Buffer-I

<table>
<thead>
<tr>
<th>For 2 L</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sodium Acetate</strong></td>
</tr>
<tr>
<td><strong>WFI</strong></td>
</tr>
</tbody>
</table>

Sodium acetate was dissolved in 1.8 L of WFI. pH was adjusted to 6.0 ± 0.2 with 1 M Acetic acid and volume was made up to 2.0 L using WFI. Filtered through 0.22 μm filter and stored at room temperature.

### 69. Acetate Buffer-II

<table>
<thead>
<tr>
<th>For 1.5 L</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sodium Acetate</strong></td>
</tr>
<tr>
<td><strong>WFI</strong></td>
</tr>
</tbody>
</table>

Sodium acetate was dissolved in 1.4 L of WFI. pH was adjusted to 3.7 ± 0.1 with 1 M Acetic acid and volume was made up to 1.5 L using WFI. Filtered through 0.22 μm filter and stored at room temperature.

### 70. Sodium Hypochlorite solution (0.1% v/v)

<table>
<thead>
<tr>
<th>For 100 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sodium Hypochlorite (4% w/v)</strong></td>
</tr>
<tr>
<td><strong>Milli-Q</strong></td>
</tr>
</tbody>
</table>

Stored at room temperature.
71. Pre Equilibration Buffer (10X) used for Gel-Filtration Chromatography

<table>
<thead>
<tr>
<th>For 200 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tri Sodium citrate dihydrate</td>
</tr>
<tr>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Citric acid</td>
</tr>
<tr>
<td>WFI</td>
</tr>
</tbody>
</table>

Ingredients were dissolved in 125 mL of WFI and pH was adjusted to 6.9 ± 0.2 with 1 M NaOH. Volume was made up to 200 mL using WFI. Filtered through 0.22 μm filter and stored at room temperature.

72. Formulation Buffer

<table>
<thead>
<tr>
<th>For 2 L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tri Sodium citrate dihydrate</td>
</tr>
<tr>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Citric acid</td>
</tr>
<tr>
<td>WFI</td>
</tr>
</tbody>
</table>

Ingredients were dissolved in 1.8 L of WFI, pH was adjusted to 6.9 ± 0.2 using 1 M NaOH / 1 M HCl and volume was made up to 2.0 L using WFI. Filtered through 0.22 μm filter and stored at room temperature.

73. Storage Buffer used for Gel-Filtration Chromatography

<table>
<thead>
<tr>
<th>For 2 L</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPA</td>
</tr>
<tr>
<td>WFI</td>
</tr>
</tbody>
</table>

Mixed and filtered through 0.22 μm filter and stored at room temperature.
Note:

1. Milli-Q water/ WFI was used for all reagents and media (if prepared from powder)

2. ‘Cell culture tested’ grade chemicals were used for cell culture experiments.

3. Media and other reagents (if required) were stored at 5 ± 3°C.

4. Buffers / solutions were prepared in 1 or 2 days in advance unless mentioned otherwise.

5. Buffers / solutions were cooled to room temperature prior to use (24 ± 2°C).

6. All open processes were carried out in Laminar flow cabinet.