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Buffer Recipes

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ABSTRACT

Recipes for a variety of buffers that I have used for my work.

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Buffer Recipes -

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500 mM HEPES-KOH (pH 7.2)

Formula Weight = 238.3 grams / mole

Recipe: Add 119.15 grams of HEPES powder to 500 mL of milliQ water. Then titrate to a pH of 7.2 with KOH and top off to a final volume of 1 liter with milliQ water.

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500 mM Tris-HCl (pH 7.6)

Formula Weight = 121.14 grams / mole

Recipe: Add 30.29 grams of Tris-Base to 300 mL of milliQ water. Then titrate to a pH of 7.6 with HCl and top off to a final volume of 500 mL with milliQ water.

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500 mM $MgCl_2$

Formula Weight = 203.31 grams / mole

Recipe: Add 25.4 grams to 250 mL of milliQ water.

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1 M Tris (untitrated)

Formula Weight = 121.14 grams / mole

Recipe: Add 30.28 grams to 250 mL of milliQ water.

2.5 M KCl

Formula Weight = 74.5513 grams / mole

Recipe: Add 186.4 grams to 1 liter of milliQ water.

Buffer S-300 (High Salt Buffer for Cation Exchange Column - eEF2 purification)

Components:

1. 20 mM HEPES-KOH, pH 7.2
   - 40 mL of 500 mM HEPES-KOH (pH 7.2)
2. 10% glycerol
   - 100 mL of 100% Ultra-Pure Glycerol
3. 5 mM $MgCl_2$
   - 10 mL of 500 mM $MgCl_2$
4. 300 mM KCl
   - 120 mL of 2.5 M KCl
5. 730 mL of milliQ water

* Add PMSF and DTT to a final concentration of 1 mM right before using Buffer S-300.

Buffer S (Initial No Salt Sample Buffer for Cation Exchange Column - eEF2 purification)

Components:

1. 20 mM HEPES-KOH, pH 7.2
   - 120 mL of 500 mM HEPES-KOH, pH 7.2
2. 10% glycerol

- 300 mL of 100% glycerol

3. 5 mM $MgCl_2$

- 30 mL of 500 $MgCl_2$

4. Top off to a final volume of 3 L with milliQ water.

* Add PSMF to a final concentration of 0.1 mM and DTT to a final concentration of 1 mM right before use.

Buffer S-20 (Low Salt Buffer for Cation Exchange Column - eEF2 purification)

Components:

1. 20 mM HEPES-KOH, pH 7.2
   - 40 mL of 500 mM HEPES-KOH, pH 7.2
2. 10% glycerol
   - 100 mL of 100% glycerol
3. 5 mM $MgCl_2$
   - 10 mL of 500 mM $MgCl_2$
4. 20 mM KCl
   - 8 mL of 2.5 M KCl
5. Top off to a final volume of 1 L with milliQ water.

*Add DTT to a final concentration of 1 mM right before using the buffer.

Buffer Q-40

Components:

1. 20 mM Tris-HCl, pH 7.6
   - 40 mL of 500 mM Tris-HCl, pH 7.6
2. 10% glycerol
   - 100 mL of 100% glycerol
3. 5 mM $MgCl_2$
   - 10 mL of 500 mL $MgCl_2$
4. 40 mM KCl
   - 16 mL of 2.5 M KCl
5. Top off to a final volume of 1 liter with milliQ water.

*Add DTT to a final concentration of 1 mM right before using the buffer.

Buffer Q-500

Components:

1. 20 mM Tris-HCl, pH 7.6
   - 40 mL of Tris-HCl, pH 7.6
2. 10% glycerol
   - 100 mL of 100% glycerol
3. 5 mM $MgCl_2$
   - 10 mL of 500 mM $MgCl_2$
4. 500 mM KCl
   - 200 mL of 2.5 M KCl
5. Top off to a final volume of 1 liter with milliQ water.

*Add DTT to a final concentration of 1 mM right before using the buffer.

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eEF2 Storage Buffer

Components:

1. 20 mM HEPES-KOH, pH 7.2
   - 10 mL of 1 M HEPES-KOH, pH 7.2
2. 1 mM $MgCl_2$
   - 1 mL of 500 mM $MgCl_2$
3. 100 mM KCl
   - 20 mL of 2.5 M KCl
3. Top off to a final volume of 500 mL with milliQ water.

*Add DTT to a final concentration of 1 mM right before using the buffer.

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eEF1A Storage Buffer

Components:

1. 20 mM Tris-HCl, pH 7.6
   - 40 mL of 500 mM Tris-HCl, pH 7.6
2. 0.1 mM EDTA
   - 200 uL of 0.5 M EDTA
3. 100 mM KCl
   - 40 mL of 2.5 M KCl
4. 25% glycerol
   - 250 mL of 100% glycerol
5. Top off to a final volume of 1 liter with milliQ water

*Add DTT to a final concentration of 1 mM right before using the buffer.

Beads-Wash Solution

Components:

1. 1 mM HCl
   - Add 41.32 uL of 12.1 M HCl to 500 mL of milliQ water.

Wash Buffer 2

Components:

1. 0.1 M Na-acetate
   - 4.1 grams of Na-acetate (F.W. = 82.03 g/mol)
2. 0.5 M NaCl
   - 14.61 grams of NaCl (F.W. = 58.44 g/mol)
3. Fill up to 300 mL of milliQ water and then adjust pH to 5.0.
4. Top off to a final volume of 500 mL with milliQ water.

Coupling Buffer

Components:

1. 0.2 M $NaHCO_3$
   - 8.4 grams of $NaHCO_3$ (F.W. = 84.007 g/mol)
2. 0.5 M NaCl
   - 14.61 grams of NaCl (F.W. = 58.44 g/mol)

3. Fill up to 300 mL of milliQ water and then adjust pH to 8.3.
4. Top off to a final volume of 500 mL with milliQ water.

Quench (Q) Buffer

Components:

1. 0.1 M Tris-HCl (pH 8.5)
   - 6.057 grams of Tris base (F.W. = 121.14 g/mol)
2. Fill up to 300 mL of milliQ water and then adjust pH to 8.5.
3. Top off to a final volume of 500 mL with milliQ water.

*When making buffers for coupling the amine-modified oligo to sepharose beads, make sure to make the Quench Buffer last, as contaminating your other buffers with the Tris that it contains will likely result in the oligo not binding to the beads properly.

Buffer HIC - A (DON'T USE THIS BUFFER - THE KCl PRECIPITATES OUT OF SOLUTION)

Components:

1. 20 mM HEPES-KOH, pH 7.2
   - 10 mL of 1 M HEPES-KOH, pH 7.2
2. 1 mM $MgCl_2$
   - 1 mL of 500 mM $MgCl_2$
3. 3.5 M KCl
   - 130.5 grams of KCl powder
4. Top off to a final volume of 500 mL with milliQ water.

*Add DTT to a final concentration of 1 mM right before using the buffer.

- This is the buffer meant to be used for my Hydrophobic Interaction Column purifications of unlabeled eEF2-YBBR from labeled eEF2-YBBR

Buffer HIC - A - NaCl

Components:

1. 20 mM HEPES-KOH, pH 7.2
   - 10 mL of 1 M HEPES-KOH, pH 7.2
2. 1 mM $MgCl_2$
   - 1 mL of 500 mM $MgCl_2$
3. 3 M NaCl
   - 87.66 grams of NaCl powder
4. Top off to a final volume of 500 mL with milliQ water.

*Add DTT to a final concentration of 1 mM right before using the buffer.

- This is the buffer meant to be used for my Hydrophobic Interaction Column purifications of unlabeled eEF2-YBBR from labeled eEF2-YBBR

Buffer HIC - B

Components:

1. 20 mM HEPES-KOH, pH 7.2
   - 10 mL of 1 M HEPES-KOH, pH 7.2
2. 1 mM $MgCl_2$
   - 1 mL of 500 mM $MgCl_2$

3. Top off to a final volume of 500 mL with milliQ water.

*Add DTT to a final concentration of 1 mM right before using the buffer.

- This is the buffer meant to be used for my Hydrophobic Interaction Column purifications of unlabeled eEF2-YBBR from labeled eEF2-YBBR
