**Buffer recipes**

**[8M Urea Sample Buffer](https://luky-my.sharepoint.com/:x:/g/personal/agwe229_uky_edu/Ec7KCbKNPQlOsfrBDK2EymIBU6sd3hlzDY0Cf3Er_LW5kA) (100mL)**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **8M Urea Buffer** | |  |  |  |  |  |  |  |  |
| Made by |  |  |  |  |  |  |  |  |  |
| Date |  |  |  |  |  |  |  |  |  |
| **Compound** | **Company** | **Lot #** | **MW** | **% purity** | **Corrected MW** | **Final Conc (M)** | **Amount for 1000mL (g/mL)** | **Amount for 100mL (g/mL)** | **Amount added** |
| Urea | Sigma-Aldrich | SLCJ4799 | 60.06 | 99.50% | 60.361809 | 8.00E+00 | 482.8945 | 48.2894 |  |
| Thiourea | Sigma-Aldrich | SLCJ4736 | 76.12 | 99.40% | 76.579476 | 2.00E+00 | 153.1590 | 15.3159 |  |
| Tris-HCl | Bio-Rad | 64380543 | 121.14 | 99.90% | 121.26126 | 5.00E-02 | 6.0631 | 0.6063 |  |
| **10% SDS** |  |  |  |  |  | **10% (w/v)** | 100.0000 | 10.0000 |  |
| DTT (Dithiothreitol) | Roche Diagnostics | 56166123 | 154.25 | 97.00% | 159.02371 | 4.00E-03 | 0.6361 | 0.0636 |  |
| 0.03% Bromophenol Blue | **(add after pH-ing)** |  |  |  |  | **0.03% (w/v)** | 0.3000 | 0.0300 |  |
|  |  |  |  |  |  |  |  |  |  |
| **Target pH = 6.80** | | \*note: stir gently on a hot plate until the solution is at room temperature and if all the solids are dissolved, then heat but do NOT heat above 40 ºC | | | | | | | |
| Initial pH |  | \*note: aliquot and store at -20 ºC | | | |  |  |  |  |
| Final pH |  |  |  |  |  |  |  |  |  |
| HCl / KOH added |  |  |  |  |  |  |  |  |  |

**[50% Glycerol w/ Protease inhibitors (100 mL)](https://luky-my.sharepoint.com/:x:/g/personal/agwe229_uky_edu/EZ_uunJ2nfJOgSR0Wa-s6aMBzt59KKxQTzFyErN6rzQ8rw)**

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| **50% Glycerol w/ Protease inhibitors** | |  |  |  |  |  |  |  |  |
| Made by |  |  |  |  |  |  |  |  |  |
| Date |  |  |  |  |  |  |  |  |  |
| **Compound** | **Company** | **Lot #** | **MW** | **% purity** | **Corrected MW** | **Final Conc (M)** | **Amount for 1000 (g/mL)** | **Amount for 100 ml** | **Amount added** |
| Glycerol |  |  |  |  |  | 50% (v/v) | 500.00 | 50 |  |
| E64 |  |  | 60.06 | 1 | 6006 | 4.00E-05 | 0.24 | 0.024024 |  |
| Leupeptin |  |  | 76.12 | 1 | 7612 | 1.60E-04 | 1.22 | 0.121792 |  |
| PMSF |  |  | 174.19 | 1 | 17419 | 2.00E-04 | 3.48 | 0.34838 |  |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
|  |  | \*note: aliquot and store at -20 ºC | | | |  |  |  |  |

**[5X Titin Buffer](https://luky-my.sharepoint.com/:x:/g/personal/agwe229_uky_edu/EcdrLCITBUpIoS4E42Fs6YcBZLvbZvBORCjq7F0zRTeJcQ)**

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| **5X Titin Buffer** | |  |  |  |  |  |  |  |  |
| Made by |  |  |  |  |  |  |  |  |  |
| Date |  |  |  |  |  |  |  |  |  |
| **Compound** | **Company** | **Lot #** | **MW** | **% purity** | **Corrected MW** | **Final Conc (M)** | **Amount for 1000 (g/mL)** | **Amount for 500 ml** | **Amount added** |
| Trizma base |  |  | 121.14 | 1 | 12114 | 2.50E-01 | 3028.50 | 1514.25 |  |
| Glycine |  |  | 75.07 | 1 | 7507 | 1.92E+00 | 14413.44 | 7206.72 |  |
| 0.5% SDS |  |  |  |  |  |  | 5.00 | 2.5 |  |
|  |  |  |  |  |  |  |  |  |  |
| **Target pH = 8.5** | |  |  |  |  |  |  |  |  |
| Initial pH |  | \*note: store at 4 ºC | | |  |  |  |  |  |
| Final pH |  |  |  |  |  |  |  |  |  |
| HCl / KOH added |  |  |  |  |  |  |  |  |  |

**3M Tris, pH 9.3 (100 mL)**

|  |  |  |  |  |  |  |  |  |  |
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| **5X Titin Buffer** | |  |  |  |  |  |  |  |  |
| Made by |  |  |  |  |  |  |  |  |  |
| Date |  |  |  |  |  |  |  |  |  |
| **Compound** | **Company** | **Lot #** | **MW** | **% purity** | **Corrected MW** | **Final Conc (M)** | **Amount for 1000 (g/mL)** | **Amount for 100 ml** | **Amount added** |
| Trizma base |  |  | 121.14 | 1 | 121.14 | 3 | 363.42 | 36.342 |  |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  | Tris base (FW: 121, pKa = 8.2 at 20˚C) |  |  |
| **Target pH = 9.3** | |  |  |  |  |  |  |  |  |
| Initial pH |  | \*note: store at room temp ºC | | |  |  |  |  |  |
| Final pH |  |  |  |  |  |  |  |  |  |
| HCl / KOH added |  |  |  |  |  |  |  |  |  |

**10% ammonium persulfate (1 ml)**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **10% APS** | |  |  |  |  |  |  |  |  |
| Made by |  |  |  |  |  |  |  |  |  |
| Date |  |  |  |  |  |  |  |  |  |
| **Compound** | **Company** | **Lot #** | **MW** | **% purity** | **Corrected MW** | **Final Conc (M)** | **Amount for 10 mL** | **Amount for**  **1 mL** | **Amount added** |
| Ammonium Persulfate |  |  |  |  |  |  | 1.0 g | 0.1 g |  |
|  |  |  |  |  |  |  |  |  |  |