**Supplementary File 1**

**HEPES Buffer**

|  |  |  |  |
| --- | --- | --- | --- |
|  | MW (g/mol) | Molarity (mM) | Grams to Add |
| NaCl | 58.44 | 200 | 2.922 |
| HEPES | 238.3 | 50 | 2.97875 |
| pH: 7.35 |  |  |  |
| Desired Volume (mL) | 250 |  |  |

**T50**

|  |  |  |  |
| --- | --- | --- | --- |
|  | MW (g/mol) | Molarity (mM) | Grams to Add |
| NaCl | 58.44 | 50 | 0.7305 |
| Tris | 121.14 | 10 | 0.30285 |
| pH: 7.35 |  |  |  |
| Desired Volume (mL) | 250 |  |  |

 **Recording Buffer (RB)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Conc. (mM) (1x) | MW (g/mol) | Weight (g) (1x) | Stock |
| NaCl | 128 | 58.44 | 3.734316 | 1 |
| KCl | 2 | 74.5513 | 0.0745513 | Volume/L |
| MgCl2 | 1.2 | 203.3027 | 0.1219816 | 0.5 L |
| CaCl2 | 2.5 | 147.0 | 0.18375 |  |
| HEPES | 10 | 238.3012 | 1.191506 |  |
| Sucrose | 10 | 342.2965 | 1.7114825 |  |
| NaOH |  |  |  | pH 7.2–7.4 |
| Osmolarity | 290.1 |  |  |  |

**Single-Molecule** **Recording Buffer (SRB.)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Conc. (mM) (1x) | MW (g/mol) | Weight (g) (1x) | Stock |
| NaCl | 128 | 58.44 | 3.734316 | 1 |
| KCl | 2 | 74.5513 | 0.0745513 | Volume/L |
| MgCl2 | 1.2 | 203.3027 | 0.1219816 | 0.5 L |
| CaCl2 | 2.5 | 147.0 | 0.18375 |  |
| HEPES | 40 | 238.3012 | 1.191506 |  |
| NaOH |  |  |  | pH 7.2–7.4 |
| Osmolarity | 311.1 |  |  |  |

**Lysis Buffer**

1. Add protease inhibitor (1 pill for 10 mL) to HEPES buffer (mix thoroughly to dissolve pellet fully)
2. Add the detergent of choice (greater than 50 times critical micelle concentration (CMC) of detergent, in this study, we used 1% DDM.
3. Mix gently, minimizing bubbles.