**Acrylamide Plug**

1. Combine in a 15 mL falcon tube:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| 1.924 | mL | ( | 962.0 | μL \* 2 ) | Deionized water |
| 1.7 | mL | ( | 850.0 | μL \* 2 ) | 50% glycerol |
| 2.12 | mL | ( | 706.7 | μL \* 3 ) | 3 M Tris (pH 9.3) |
| 2.72 | mL | ( | 906.7 | μL \* 3 ) | 30% bis-Acrylamide |
| 24 | μL |  |  |  | 10% APS |

1. Mix by gently pipetting a few times**.**
2. Add **13 µL of TEMED** (tetramethyl ethylenediamine). Mix by gently pipetting a few times**.**

**1% Agarose gel**

1. Combine in a beaker:

|  |  |  |
| --- | --- | --- |
| 16.0 | mL | 5X Titin Buffer |
| 15.7 | mL | Deionized water |
| 48.3 | mL | 50% glycerol\* |
| 0.8 | g | SeaKem Gold Agarose\*\* |

\*Glycerol is included in the mixture to increase the solution viscosity inside the gel and thus sharpen the protein bands.

\*\*It is essential to use SeaKem Gold agarose for optimal migration of high molecular weight proteins. This type has large pore size and excellent mechanical stability. Other types of Agaroses may be used, but the protein mobility will be significantly reduced.

1. Add stir bar to beaker, and weight / record the weight of the beaker
   * This weight will be used so you can replace the water that lost during heating
2. Place the beaker on the hot plate and stir at 100 rpm / heat to 250 °C
   * Place a small beaker with 50 mL of water next to it. You will use this to replace any lost water.
   * Be careful to watch and make sure the agarose does not boil over
   * Using the stir bar during the heating step eliminates non-hydrated agarose granules in the final gel.

**10% SDS-Polyacrylamide Resolving Gel (5.0 mmol/L PhosTag)**

1. Combine in a 15 mL falcon tube:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| 4.48 | mL | ( | 896.0 | μL \* 5 ) | Deionized water |
| 2.50 | mL | ( | 833.3 | μL \* 3 ) | 1.5 M Tris (pH 8.8) |
| 2.67 | mL | ( | 890.0 | μL \* 3 ) | 30% bis-Acrylamide |
| 100 | μL |  |  |  | 10% SDS (w/v) |
| 100 | μL |  |  |  | 5.0 mmol/L PhosTagTM AAL Solution |
| 100 | μL |  |  |  | 10 mmol/L MnCl2 Solution |
| 50 | μL |  |  |  | 10% APS (w/v) |

1. Mix by gently pipetting a few times**.**
2. Add **10 µL of TEMED** (tetramethyl ethylenediamine). Mix by gently pipetting a few times**.**
3. **Add 5 mL to each gel.**

\*Red is for PhosTag gels only\*

**4.5% SDS-Polyacrylamide Stacking Gel**

1. Combine in a 15 mL falcon tube:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| 4.48 | mL | ( | 803.3 | μL \* 3 ) | Deionized water |
| 100 | μL |  |  |  | 0.5 M Tris (pH 6.8) |
| 534 | μL |  |  |  | 30% bis-Acrylamide |
| 100 | μL |  |  |  | 10% SDS (w/v) |
| 20 | μL |  |  |  | 10% APS (w/v) |

1. Mix by gently pipetting a few times**.**
2. Add **8 µL of TEMED** (tetramethyl ethylenediamine). Mix by gently pipetting a few times**.**
3. **Fill to top of gel caster and then add combs in quickly**