

SOP: R010

Western Blot Transfer Buffer

Materials and Reagents:

1. Clean 2L White -Top Nalgene Container (Cat.# Fisher 02-963-2A)
2. 1L Graduated Cylinder
3. 250 ml Graduated Cylinder
4. Stir-Bar
5. Magnetic Stir-Plate
6. Magnetic Stir-Bar retriever
7. 800 ml Milli-Q H₂O
8. 3.03 g Tris-base (also called Trizma Base)
9. 14.4 g glycine
10. 200 ml methanol

Protocol: (note 1)

1. ____ Clean the Transfer Buffer container and rinse with Milli-Q H₂O (note 2).
2. ____ Measure 800 ml of Milli-Q H₂O with the 1L Graduated Cylinder.
3. ____ Add the Milli-Q H₂O to the Transfer Buffer container.
4. ____ Weigh 3.03 g of Tris-base (Trizma-base).
5. ____ Add the Tris-base to the 800 ml of Milli-Q H₂O.
6. ____ Place magnetic Stir-Bar into the container and mix using Stir-Plate.
7. ____ Weigh 14.4 g of glycine.
8. ____ When the Tris-base is dissolved, add the glycine and dissolve.
9. ____ With the 250 ml graduated cylinder, measure 200 ml of methanol (note 3).
10. ____ Add methanol to the mixture and continue stirring for 15 minutes.
11. ____ Use magnetic Stir-Bar retriever to remove Stir-Bar.
12. ____ Ensure that the container is labeled "Western Blot Transfer Buffer" and write your initials and the date on a piece of tape and place on container.

Notes:

1. Always make sure to wear gloves when preparing buffers.
2. The Nalgene carboy (Fisher Catalog # 02-963-2A) used for this buffer should be dedicated for this purpose.
3. Use caution when measuring solvents such as methanol, avoid skin contact.