

PROTOCOL FOR DIALYZING NUCLEAR NER LYSATES**DAY BEFORE:**

1. You will need 1000ml of sterile filtered HNM buffer per cassette to dialyze each sample.

For 1 Liter:

*Use Rnase/Dnase free Water. Add Hepes first, then pH to 7.5 and add the final reagents.

	<i>MW</i>	<i>Final Concentration</i>	<i>Amount</i>
<i>Hepes pH to 7.5</i>	238.3g	50mM	11.9g
<i>NaCl</i>	58.44	150mM	8.77
<i>MgCl₂</i>	(1M)	1mM	1ml

2. DEPC treat and autoclave a 500 or 600ml beaker and stir bar for each nuclear lysate that will be dialyzed.

PROCEDURE:

1. The Pierce Slide-A-Lyzer Dialysis Cassettes (Cat. # 66380) will hold 0.5 – 3ml of lysate. They must be hydrated before use, so remove the cassette from the pouch and slip into a buoy. Immerse the cassette in dialysis buffer (HNM buffer) for 30 seconds.
2. Remove the cassette from the buffer and tap the edge gently on paper towels to remove excess liquid from the cassette. **DO NOT BLOT THE MEMBRANE.**
3. When adding the sample, do not allow the needle to contact the membrane.
4. Using a 20 gauge needle, fill the syringe with your sample and leave a small amount of air in the syringe.
5. With the bevel of the needle sideways, insert the tip into a syringe port at a top corner (**be sure to mark this port so you will know where the sample was loaded**).
6. Inject the sample slowly allowing the beveled portion of the needle to penetrate the gasket minimally (be careful not to overextend the needle which will puncture the membrane).
7. While the needle is still inserted into the gasket, draw up on the syringe to remove air from the cassette cavity, compressing the membrane so the sample contacts the greatest window surface area.
8. Remove the needle from the cassette and the gasket will reseal itself, allowing no direct air contact.
9. Slip the cassette into the groove of a flotation buoy and float the assembly in a 600ml DEPC treated beaker with a DEPC treated stir bar in about 500ml of the HNM buffer.
10. Allow the sample to dialyze for 2 hours at room temperature on a stir plate.
11. Change the dialysis buffer and allow the sample to dialyze overnight in the 4°C room on a stir plate.
12. To remove the sample after dialysis, fill the syringe with a volume of air equal to your sample volume and penetrate the gasket with the needle through a top unused port. Expel the air into the membrane cavity so as not to collapse the membranes when extracting the sample.
13. Rotate the cassette until the port with the syringe is on the bottom and draw back on the syringe to remove the dialyzed sample.
14. Remove the needle from the cassette and discard the cassette.
15. Your sample is now ready to be quantified using the BCA (Pierce) Protein Assay.
16. Store at -80°C.