

Appendix 7.

Buffers Used:

1.-AP Buffer: 100 mM NaCl , 5mM MgCl₂, 100 mM Tris p.h. 9.5.

5M NaCl: Dissolve 292.2 g of NaCl in 800 mL of water. Adjust the volume to 1 liter with water. Dispense into aliquots and sterilize by autoclaving.

1M MgCl₂ : Dissolve 203.3 g of MgCl₂, adjust the volume to 1 liter of water. Dispense into aliquots and autoclave.

1M Tris: 121g 1000 mL.

2.- Gel Loading Buffer (agarose gel) 6x: 0.25% bromofenol blue, 0.25% xylene cyanol FF, 40% water.

3.-1.5 Tris (PH 8.8): 90.82 gr Tris in 1000 mL water

4.-1M Tris (pH6.8): 60.55 gr Tris in 1000 mL water.

5.-10% SDS: 10 gr SDS in 90 mL water. Dissolve by keeping at 68°C and then adjust the pH to 7.2 adding few drops of concentrated HCl. Make the volume 100 mL by adding water.

6.-Crystal Violet (for stainin monolayer of cells): For staining monolayers of cells, 1 g crystal violet, 100 mL EtOH, 400 mL water.

7.-8% Formaldehyde (for fixing cells): 108.1 MI of 37% formaldehyde.

8.-Protein Transfer Buffer (Western Blot): 3.03 Trizma Base, 2 gr of Glycine, 200 mL of methanol. Add 650 of distilled water, dissolve, make final volume up to 1000 mL. Store at 2-8 °C.

8.- RIPA Buffer (for Co-IP): 300 mM NaCl (1.5 mL), 1.0% NP40 (250 μ L), 0.5% DOC (sodium salt) (0.125gr), 0.1% SDS (5mL), 50 mM Tris PH 8 (1250 μ L). Add sigma cocktail inhibitor before use (50 microliters for 1 Lt).

9.-PBS: 80g NaCl, 2 gr KCl,14.4gr Na₂PO₄, 2.4 g KH₂PO₄.

10.-TBST (10x) 1 Lt PH 7.4: 500 mM NaCl, 20mM Tris PH 7, 0.2% Tween.

11.- Methyl cells (2%) (for plaque assays): in a Pyrex Bottle 36g of methyl cellulose 4,000 cP autoclave wrapped in aluminum foil, 900 mL of water. Autoclave in 1.8 L Pyrex Bottle with stir bar. In sterile conditions add methylcell to the distilled water. Stir continuously. For 2x DMEM add 900 mL of sterile water and 24.26 gr of DMEM. Filter, sterilize and add to the methyl cells.

12.-50x TAE 242 g Tris Base, 57.1 mL acetic acid, 100mL 0.5 EDT (PH 8.0)

13.- Running Buffer (Western Blot): 0.375M Tris, 1.92 Glycine, 0.1% SDS.