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BUFFERS, SOLUTIONS AND CULTURE MEDIA:

PLEASE PAY ATTENTION TO THE RECIPES WITH ANHYDROUS VS. HYDRATED SALTS!!!

General solutions

- **1M TrisHCL (1MT):** 121.14 g Tris base in 900 ml ddH_2O. Adjust pH to 7.4-7.6 with concentrated HCl and complete volume to 1000 ml

- **5 M NaCl (5MN):** 292.25 g NaCl in 1000 ml ddH_2O. **Do not adjust pH.** This solution filters very slowly through Whatman filter and does not filter well through sterile filters if they have not been pre-wetted with ddH_2O

- **2.5 M NaCl (2.5MN):** 146.13 g NaCl in 1000 ml ddH_2O. **Do not adjust pH.**

- **1X TBS:** 50 mM TrisHCl + 150 mM NaCl, pH 7.4-7.6
  Use 50 ml 1MT + 30 ml 5MN per liter

- **TBS-Tween low salt:** 50 mM TrisHCl + 150 mM NaCl, pH 7.4-7.6 + 0.2 % v/v Tween-20
  Use 50 ml 1MT + 30 ml 5MN + 2 ml 100% Tween20 (or 10 ml 20% Tween20) per liter

- **TBS-Tween high salt:** 50 mM TrisHCl + 500 mM NaCl, pH 7.4-7.6 + 0.2 % v/v Tween-20
  Use 50 ml 1MT + 100 ml 5MN + 2 ml 100% Tween20 (or 10 ml 20% Tween20) per liter

- **Phosphate buffer: (4XPB)**
  Monobasic phosphate, anhydrous: NaH_2PO_4 119.90 g/mol
  Dibasic phosphate, anhydrous: Na_2HPO_4 141.96 g/mol

To prepare 500 ml of 0.4M PB **of pH 7.4**: measure 23.0 g Na_2HPO_4 + 4.56 g NaH_2PO_4 into 450 ml ddH_2O. Mesure pH (should be very close to 7.4) and adjust very slightly with solid phosphate salts or diluted HCl. This solution must be filtered through Whatman filter and cannot be stored in the cold (it crystallizes)

To prepare 500 ml of 0.4M PB of a different pH, first prepare 500 ml of 0.4 M solution for each phosphate salt:
- 23.98 g NaH_2PO_4/500 ml (do not adjust pH)
- 28.39 g Na_2HPO_4/500 ml (do not adjust pH)

Then, mix the following volumes under constant pH measurement:

pH at 25 °C	0.4M Na_2HPO_4	0.4M NaH_2PO_4
5.8	40.0 ml	460.0 ml
6.0	61.5	438.5
6.2	92.5	407.5
6.4	132.5	367.5
6.6	187.5	312.5
6.8	245.0	255.0
7.0	305.0	195.0
7.2	360.0	140.0
7.4	**405.0**	**95.0**
7.6	435.0	65.0
7.8	457.5	42.5
8.0	473.5	26.5

- **50 mM PBS:** 50 mM sodium phosphate + 150 mM NaCl, pH 7.4
  Mix 125 ml 4XPB + 30 ml 5MN in 1000 ml ddH_2O. Verify pH and filter.

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PBS (Harlow & Lane)

10 mM PB with 150mM Cl^-

NaCl	8 g
KCl	0.2 g
Na_2HPO_4	1.44 g (anh)
KH_2PO_4	0.24 g (anh)

Dissolve in 900 ml ddH_2O. Adjust pH to 7.4 with diluted NaOH or HCl, and complete to final vol= 1 lt. Filter through Whatman filter and store at 4C or room temp.

Immunochemistry:

- **4% PFA in PB:** 4% Paraformaldehyde in 10 mM phosphate buffer (pH 7.4) for cell/tissue fixation. Microwave ~ 50 ml ddH2O for 45 sec. Add 1 drop of concentrated NaOH to make it strongly alkalyne, and measure temperature after microwaving until temp. goes down to ~55-60 °C. Weigh 1.6 g PFA (granular) in a 50 ml Falcon, add 30 ml of warm H_2O and vortex vigorously until dissolved. Complete volume with 10 ml of 4XPB at pH 7.4. Filter through Whatman and use immediately. Store leftover in the cold for no more than a week and use leftover only for cells (make fresh for tissue).

- **$NaBH_4$:** Sodium borohydride 0.2% (a.k.a. sodium tetrahydroborate) is used to quench autofluorescence in tissue sections. This reagent is highly flammable in contact with moisture and is very toxic to the skin. Do not leave the flask uncapped. Close it tightly after weighing, seal with parafilm, wrap it in its non-flammable cloth and return to its original metal canister. Preparation:
  0.2 g $NaBH_4$ + 25 ml 0.4 M phosphate buffer pH 7.4 + 75 ml dd H_2O

- **Citrate buffer:** For antigenic recovery. 10 mM sodium citrate, pH 6-6.5 adjusted with HCl.

Protein electrophoresis and Western blotting:

- **10X Running buffer: (WITHOUT SDS)** For **protein PAGE**: 250 mM Tris + 1.92 M Glycine, pH 8.0-8.3
  30.3 g Tris base + 144.2 g Glycine in 1000 ml ddH_2O. Measure pH but do not adjust (if it's not in the appropriate range, discard the solution). Filter through sterile filter and store in the cold (bacteriae can grow on glycine-based buffers). **Do not add SDS!**

- **Running buffer:** For SDS-PAGE: 25 mM Tris + 192 mM Glycine + 0.1 % w/v SDS (pH 8.0-8.3)
  3.03 g Tris base + 14.43 g Glycine in 995 ml ddH_2O + 5 ml 20% w/v SDS. Do not adjust pH and use immediately. Store leftover in the cold and use within a week.
  Alternatively: 100 ml 10X running buffer + 5 ml 20% SDS in 1 liter ddH_2O.

- **Towbin buffer:** For PAGE transfer: 25 mM Tris + 192 Glycine + 20 % v/v Methanol (pH 8.0-8.3)
  3.03 g Tris base + 14.43 g Glycine in 800 ml ddH_2O + 200 ml Methanol. Do not adjust pH and use immediately. Discard any leftover.
  Alternatively: 100 ml 10X running buffer + 200 ml Methanol in 1 liter ddH_2O.
  Add SDS to 0.1% final only for very high MW proteins and only if needed.

- **Resolving buffer: (TrisHCl gels)** 1.5M TrisHCl, pH 8.8
  90.86 g Tris base in 500 ml ddH_2O. Adjust pH with concentrated HCl and filter through Whatman paper

- **Stacking buffer: (TrisHCl gels)** 0.5 M TrisHCl, pH 6.8
  30.29 g Tris base in 500 ml ddH_2O. Adjust pH with concentrated HCl and filter through Whatman paper

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- **Layover solution: (TrisHCl gels)** 10 ml Resolving buffer + 70 ml Ethanol + 20 ml ddH_2O.

- **Tris-Tricine anode:** Buffer for the anode (outer compartment) in Tris-Tricine gels
  200 mM TrisHCl, pH 8.9
  (24.22 g Tris base / 1 lt ddH_2O, adjust pH with concentrated HCl)

- **Tris-Tricine cathode:** Buffer for the cathode (inner compartment) in Tris-Tricine gels
  100 mM Tris, Tricine, 0.1 %w/v SDS
  (12.11 g Tris base + 17.92 g Tricine + 5ml 20% SDS / 1 lt ddH_2O)
  Do not adjust pH!! it should be ~8.0 - 8.5. Store at 4C

- **4X Laemmli buffer: (non-reducing)** 8 ml stacking buffer (0.5 M TrisHCl, pH 6.8)
  8 ml pure glycerol
  1.6 g SDS (solid)
  - complete to 20 ml with ddH_2O
  - for TrisHCl gels, add 4ul of concentrated Bromophenol blue (2% in ddH_2O)
  - for TrisTricine gels, add 10 ul of concentrated CoomassieBlue G250 (0.5% in ddH_2O)

- **3X Laemmli buffer/BME: (reducing)** 750 ul 4X Laemmli + 250 ul pure Beta-mercaptoethanol
  Mix 2 vol sample + 1 vol Laemmli, boil at 100C x 5 min

- **3X Laemmli buffer/DTT: (reducing)** 750 ul 4X Laemmli + 250 ul 1.2 M Dithiotreitol (DTT)
  Mix 2 vol sample + 1 vol Laemmli, heat at 37C x 5-10 min
  Use this buffer with samples that cannot be boiled
  Prepare stock DTT in ddH_2O and heat at 42C to dissolve it. Freeze at -20C in aliquots

- **Carbonate buffer: (for ECL)** 100 mM carbonate/bicarbonate buffer pH 9.1-9.5
  Dissolve 1.59 Na_2CO_3 (anhydrous) + 2.95 g $NaHCO_3$ (anhydrous) in 500 ml ddH_2O. To adjust pH within the desired range, add minute amounts of Na_2CO_3 (to raise pH) or $NaHCO_3$ (to lower pH) and measure pH while the salts dissolve. Filter through Whatman paper and store in the dark.

- **Alk-Phos buffer: (for W-blot)** 100 mM TrisHCl, 100 mM NaCl, 5 mM MgCl2, pH=9.0
  12.11 g Tris base + 5.84 g NaCl + 1.02 g $MgCl_2(6xH_2O)$ in 900 ml ddH_2O
  Adjust pH down to 9.0 with concentrated HCl and complete to 1000 ml.
  Filter through Whatman paper

- **5% NBT: (for W-blot)** - Prepare 8.4 ml pure DMF + 3.6 ddH_2O (➔ 70 % DMF)
  - Dissolve 500 mg NBT (whole bottle) in 10 ml of 70% DMF
  - Aliquot in 1 ml aliquots and store at -20C

- **5% BCIP: (for W-blot)** - Dissolve 500 mg BCIP (whole bottle) in 10 ml pure DMF
  - Aliquot in 1 ml aliquots and store at -20C
  - WATCH OUT that you are using "BCIP toluidine" and not BCIP sodium salt, which is not soluble in DMF

- **NBT and BCIP** These two must be prepared in dimethylformamide (DMF),
  CHECK THE CONICAL TUBES FOR RESISTANCE TO DMF.
  Many types of conical tubes will dissolve or turn white by DMF (same for plastic pipettes).
  The proportion of **NBT+BCIP and Alkaline Phosphatase buffer** for

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developing Western blots is: 10 ml AlkPhos buffer + 70 µl 5% NBT + 35 µl 5% BCIP (mix and develop within 20 min)

DNA electrophoresis:

- **20X SSC:** 3 M NaCl + 0.3M sodium citrate
  (87.68 g NaCl + 44.11 g sodium citrate dihydrate) / 500 ML ddH_2O

- **1X TE buffer:** 10 mM Tris + 1 mM EDTA, pH 7.6-8.0
  10 ml of 1 M TrisHCl (pH 7.6-8.0) + 2 ml 500 mM EDTA (pH 8.0), in 1 lt ddH_2O. Filter through Whatman paper or sterilize by autoclaving.

- **50X TAE buffer:** Buffer for DNA electrophoresis in agarose gels
  242 g Tris + 57.1 ml glacial acetic acid + 100 ml 0.5 M EDTA (pH 8.0), in 1 lt ddH_2O. Filter through Whatman paper or sterilize by autoclaving.

Deglycosylation:

- **5X CHAse buffer:** Buffer for chondroitinase ABC.
  200 mM TrisHCl, + 200 mM NaAcetate, pH 7.6 - 8.0
  12.11 g Tris base + 82.03 g Sodium acetate anhydrous, per 500 ml ddH_2O.
  Adjust pH with concentrated HCl and filter sterile.

- **16X DG buffer:** 400 mM Sodium phosphate mono/dibasic, pH 7.4 (listed as 4XPB)
  This buffer is compatible with O-glycanase, neuraminidase and N-glycanase (PNGase F) but the enzymes lose activity over time (long incubations)

Culture media (all media must be stored in the dark and at 4 degrees):

- **"DMEM"** Dulbecco's Modified Eagle Medium, suitable for most human glioma cell lines.
  Formulation: DMEM (high glucose) + 10 % fetal bovine serum (heat inactivated) + 50 U/ml penicillin + 50 U/ml streptomycin.

Suppliers: DMEM high gluc, w/Gln, wo/sodium Pyr: Invitrogen #11965 or Hyclone # 12-741F

Cheaper alternative: -DMEM powder, high gluc, w/Gln (Sigma D5648)
-dissolve in 1 lt cell culture H_2O
-add 3.7 g Na_2HCO_3
-verify or adjust pH to 7.2-7.4
-filter sterile

- **"RPMI"** RPMI-1640, suitable for CNS-1 rat glioma cell line
  Formulation: RPMI1640/glutamine + 10 % fetal bovine serum (heat inactivated) + 50 U/ml penicillin + 50 U/ml streptomycin.

Suppliers: RPMI-1640 w/Gln: Invitrogen #11875 or HyClone #12-702F

- **Oli-neu medium** For culture of the oligo precursor cell line Oli-Neu. These cells were created by selecting with G-418 a clone overexpressing the oncogene Neu.
  Formulation: Similar to Sato's medium (see below)

Base media: DMEM high glucose (Invitrogen #11965 or Hyclone # 12-741F)

Supplements:

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Supplement	Stock		final concentration		For 500 ml base media add:
Insulin	1	mg/ml in water	0.01	mg/ml	5 ml
Sodium selenite	0.038	mg/ml in water	0.038	µg/ml	500 µl
Putrescine	1.61	mg/ml in water	4.025	µg/ml	1.25 ml
Progesterone	0.62	mg/ml in ethanol	0.062	µg/ml	50 µl
Tri-Iodo-tyronine (TIT)	3.40	mg/ml in ethanol	0.34	µg/ml	50 µl
Transferrin	10	mg/ml in water	0.01	mg/ml	500 µl
Thyroxine	0.62	mg/ml in alk. EtOH	0.403	µg/ml	325 µl
Horse Serum	100%	-----	1%	-----	5 ml
G-418	50	mg/ml in water	250	µg/ml	250 µl

This medium is similar to Bottenstein and Sato's medium and, except for the TIT and thyroxine, all other components can be replaced by 1X N2 supplement (Invitrogen #17502, concentrations are not identical but will work well)
- Remove 13 ml of base medium before adding the supplements.
- Prepare insulin (50 mg) in 50 ml H2O with 100 µl 1N HCl
- Prepare Thyroxine in alkaline ethanol: 0.104 NaOH in 6 ml H2O + 14 ml ethanol
(final solution will be in 70 % ethanol with 0.13 N NaOH)

- **Neural Stem Cells:** This medium is for rodent P1 neural stem cells:
  - 480 ml DMEM/F-12
  - 5 ml L-glutamine (100x, 200 mM)
  - 5 ml N2 supplement (100x) (Invitrogen #17502)
  - 5 ml Pen/Strep (100x) and 5 ml Amphotericine (100x)
  - 10 µl human EGF (stock 1 mg/ml); final concentration: 20ng/ml

Suppliers: human EGF: Invitrogen #13247 or Peprotech #100-15
Use the medium within 2 weeks before EGF degrades. Many laboratories are currently replacing N2 supplement with B27 supplement (Invitrogen #17504).

- **Glioma stem cells:** This formulation maintains the cells in an undifferentiated state and with high proliferation rate.
  - 500 ml Neurobasal (Invitrogen #21103)
  - 10 ml B27 supplement (Invitrogen #17504)
  - 5 ml L-glutamine (100x: 200 mM)
  - 5 ml Penicillin/Streptomycin (100x)
  - 25 µl human EGF (1 mg/ml, Invitrogen or Peprotech), final conc: 50 ng/ml
  - 250 µl human bFGF (100 µg/ml, Invitrogen or Peprotech), final concentration: 50 ng/ml
  - 50 µl human LIF (100 µg/ml, GenScript), final concentration: 10 ng/ml

This formulation is expensive and can be replaced with the following one below:

- **Glioma stem cells II:** This is the formulation from Lee et. al. (Cancer Cell 2006) that has become standard in the field.
  - 500 ml DMEM/F12 (Mediatech CellGro MT10090CV)
  - 10 ml B27 supplement
  - 5 ml L-glutamine (100x: 200 mM)
  - 5 ml Penicillin/Streptomycin (100x)
  - 10 µl human EGF (1 mg/ml), final concentration: 20 ng/ml
  - 100 µl human bFGF (100 µg/ml), final concentration: 20 ng/ml