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Base RIPA Buffer

Lysis Buffers

From: Western Blot protocol

Volume: 100 mL **Storage:** 4°C for up to 6 months

Ingredients

Component	Final Concentration	Amount
Tris-HCl pH 7.6	25 mM	**2.5 mL of 1 M stock**
NaCl	150 mM	**3 mL of 5 M stock**
Sodium Deoxycholate	1% (w/v)	**1 g**
SDS	0.1% (w/v)	**0.1 g**

Component	Final Concentration	Amount
NP-40 (Igepal CA-630)	1% (v/v)	**1 mL**
Na_3VO_4 (Sodium orthovanadate)	1 mM	**100 µL of 1 M stock**
ddH_2O	-	**to 100 mL**

Preparation

1. Add ~80 mL ddH_2O to beaker with stir bar
2. Add Tris-HCl and NaCl stock solutions
3. Add sodium deoxycholate powder, stir until dissolved
4. Add SDS powder, stir until dissolved (will foam - this is normal)
5. Add NP-40, mix well
6. Add Na_3VO_4 (phosphatase inhibitor), mix well
7. Adjust pH to 7.6 if needed
8. Bring to 100 mL final volume with ddH_2O
9. Store at 4°C

> **Important Notes**
> Do NOT add protease inhibitors until day of use. Add 25X protease inhibitor and 20X phosphatase inhibitor on the day of experiment.

lysis RIPA protein extraction western blot

Strong RIPA Buffer

Lysis Buffers

From: Western Blot protocol

Volume: 100 mL **Storage:** 4°C for up to 6 months

Ingredients

Component	Final Concentration	Amount
Tris-HCl pH 7.4	50 mM	**5 mL of 1 M stock**
NaCl	150 mM	**3 mL of 5 M stock**
Sodium Deoxycholate	1% (w/v)	**1 g**
SDS	0.1% (w/v)	**0.1 g**
Triton X-100	1% (v/v)	**1 mL**
EDTA	0.5 mM	**100 µL of 0.5 M stock**
Na_3VO_4	1 mM	**100 µL of 1 M stock**
NaF	50 mM	**210 mg**
ddH_2O	-	**to 100 mL**

Preparation

1. Add ~80 mL ddH_2O to beaker with stir bar
2. Add all stock solutions (Tris, NaCl, EDTA, Na_3VO_4)
3. Add sodium deoxycholate, stir until dissolved
4. Add SDS, stir until dissolved
5. Add Triton X-100, mix well
6. Add NaF powder, stir until dissolved
7. Adjust pH to 7.4
8. Bring to final volume with ddH_2O

Important Notes

Use for difficult-to-lyse samples, tissues, or nuclear proteins. Contains built-in phosphatase inhibitors (Na_3VO_4 and NaF). Stronger than standard RIPA due to higher Tris concentration and Triton X-100.

lysis RIPA strong tissue nuclear proteins

25X Protease Inhibitor Stock

Lysis Buffers

From: Western Blot protocol

Volume: 4-8 mL **Storage:** -20°C for 1 year (aliquoted)

Ingredients

Component	Final Concentration	Amount
cOmplete™ EDTA-free tablets	1 tablet / 2 mL	**2-4 tablets**
Autoclaved water	-	**4-8 mL**

Preparation

1. Add 1 cOmplete™ tablet per 2 mL water (e.g., 4 tablets + 8 mL water)
2. Vortex vigorously until fully dissolved
3. Aliquot 200-400 µL into 1.5 mL tubes
4. Label: "25X Protease Inhibitor" + date
5. Store at -20°C

> **Important Notes**
> Use one aliquot per experiment. Do NOT refreeze after thawing! Add to RIPA buffer at 1:25 dilution (e.g., 40 µL per 1 mL RIPA).

protease inhibitor cOmplete stock solution

20X Phosphatase Inhibitor Stock

Lysis Buffers

From: Western Blot protocol

Volume: 1 mL **Storage:** -20°C for 1 year (aliquoted)

Ingredients

Component	Final Concentration	Amount
PhosSTOP™ tablets	2 tablets / 1 mL	**2 tablets**
Autoclaved water	-	**1 mL**

Preparation

1. Add 2 PhosSTOP™ tablets per 1 mL water
2. Vortex vigorously until fully dissolved
3. Aliquot 50 µL into 1.5 mL tubes
4. Label: "20X Phosphatase Inhibitor" + date
5. Store at -20°C

> **Important Notes**
> Use one aliquot per experiment. Do NOT refreeze! Add to RIPA buffer at 1:20 dilution (e.g., 50 µL per 1 mL RIPA).

phosphatase inhibitor | PhosSTOP | stock solution

10X Tris-Glycine-SDS Running Buffer

Electrophoresis

From: Western Blot protocol

Volume: 1 L **Storage:** Room temperature indefinitely

Ingredients

Component	Final Concentration	Amount
Tris base	0.25 M	**30.3 g**
Glycine	1.92 M	**144 g**
SDS	1% (w/v)	**10 g**
ddH_2O	-	**to 1 L**

Preparation

1. Add ~800 mL ddH_2O to beaker
2. Add Tris base and glycine, stir until dissolved
3. Add SDS, mix well
4. Bring to 1 L with ddH_2O
5. Store at room temperature

> **Important Notes**
> Dilute 1:10 for use (100 mL 10X buffer + 900 mL water = 1 L 1X running buffer). Do NOT adjust pH.

SDS-PAGE | running buffer | gel electrophoresis

5X SDS Loading Dye (with β-mercaptoethanol)

Electrophoresis

From: Western Blot protocol

Volume: 10 mL **Storage:** -20°C for 6 months (without βME); add βME fresh

Ingredients

Component	Final Concentration	Amount
1.5 M Tris pH 6.8	-	**3.75 mL**
20% SDS	-	**1.5 mL**
100% Glycerol	-	**3 mL**
ddH_2O	-	**1.25 mL**
Bromophenol Blue	-	**~5 mg (pinch)**
β-mercaptoethanol (add before use)	5% (v/v)	**0.05 mL per 0.95 mL aliquot**

Preparation

1. Combine 1.5M Tris pH 6.8, 20% SDS, and glycerol
2. Add ddH_2O and mix well
3. Add Bromophenol Blue until deep blue color
4. Aliquot 950 µL portions
5. Store base dye at -20°C
6. Add 50 µL β-mercaptoethanol to each aliquot before use (in fume hood)

> **Important Notes**
>
> HAZARD: β-mercaptoethanol is toxic! Work in fume hood when adding. Add to samples at 1:5 ratio (e.g., 5 µL dye per 20 µL sample). Heat samples at 95°C for 5 min after adding.

loading dye | reducing agent | beta-mercaptoethanol | SDS

Transfer Buffer (Towbin)

Transfer & Blocking

From: Western Blot protocol

Volume: 1 L **Storage:** 4°C for 1 month

Ingredients

Component	Final Concentration	Amount
Tris base	25 mM	**3.03 g**
Glycine	192 mM	**14.4 g**
Methanol	20% (v/v)	**200 mL**
ddH_2O	-	**to 1 L**

Preparation

1. Add ~600 mL ddH_2O to beaker

2. Add Tris base and glycine
3. Stir until completely dissolved
4. Add 200 mL methanol
5. Bring to 1 L with ddH_2O
6. Store at 4°C

> **Important Notes**
> Do NOT add SDS to transfer buffer. Keep cold during transfer. Methanol helps protein binding to membrane but reduces transfer of large proteins (>100 kDa).

transfer buffer | western blot | Towbin

10X Tris-Buffered Saline with Tween (TBS-T)

Transfer & Blocking

From: Western Blot protocol

Volume: 1 L **Storage:** Room temperature for 6 months

Ingredients

Component	Final Concentration	Amount
Tris base	500 mM	**60.57 g**
NaCl	1.5 M	**87.6 g**
ddH_2O	-	**~900 mL**
HCl (conc.)	-	**to adjust pH to 7.8**
Tween-20	1%	**10 mL (add to 1X)**

Preparation

1. Add ~800 mL ddH_2O to beaker
2. Add Tris base and NaCl

3. Stir until dissolved
4. Adjust pH to 7.8 with concentrated HCl
5. Bring to 1 L with ddH_2O
6. For 1X TBS-T: Dilute 100 mL 10X TBS with 890 mL water and 10 mL Tween-20

> **Important Notes**
> The 10X stock does NOT contain Tween-20. Add Tween-20 only when making 1X working solution.

TBS-T | washing buffer | blocking buffer

5% Milk Blocking Buffer

Transfer & Blocking

From: Western Blot protocol

Volume: 50 mL **Storage:** 4°C for 1 week

Ingredients

Component	Final Concentration	Amount
Non-fat dry milk powder	5% (w/v)	**2.5 g**
1X TBS-T	-	**50 mL**

Preparation

1. Add 2.5 g milk powder to 50 mL 1X TBS-T
2. Vortex or stir until completely dissolved
3. Store at 4°C

> **Important Notes**
> Use for most antibodies. Do NOT use for phospho-antibodies (use 5% BSA instead). Milk contains casein kinases that can interfere with phospho-detection.

blocking buffer milk TBS-T

5% BSA Blocking Buffer

Transfer & Blocking

From: Western Blot protocol

Volume: 50 mL **Storage:** 4°C for 1 week

Ingredients

Component	Final Concentration	Amount
BSA (Bovine Serum Albumin)	5% (w/v)	**2.5 g**
1X TBS-T	-	**50 mL**

Preparation

1. Add 2.5 g BSA to 50 mL 1X TBS-T
2. Stir gently to dissolve (avoid foaming)
3. Store at 4°C

Important Notes

Required for phospho-specific antibodies. More expensive than milk but necessary to avoid background with phospho-detection.

blocking buffer BSA phospho-antibodies

PCR Master Mix (Genotyping)

PCR & Molecular

From: Mouse Genotyping protocol

Volume: Per reaction (25 µL) **Storage:** Make fresh; keep on ice

Ingredients

Component	Final Concentration	Amount
2X GoTaq Green Master Mix	1X final	**12.5 µL**
Forward Primer (10 µM)	0.4 µM final	**1 µL**
Reverse Primer (10 µM)	0.4 µM final	**1 µL**
Nuclease-free water	-	**8.5 µL**
Template DNA	-	**2 µL**

Preparation

1. Thaw 2X GoTaq Master Mix on ice
2. Calculate total reactions needed + 10% extra
3. Prepare master mix without template DNA
4. Vortex and briefly spin down
5. Aliquot 23 µL per PCR tube
6. Add 2 µL template DNA to each tube

> **Important Notes**
> GoTaq Green contains buffer, dNTPs, Mg^{2+}, and Taq polymerase. The green dye allows direct loading on agarose gel.

PCR genotyping GoTaq master mix

50X TAE Buffer

PCR & Molecular

From: Mouse Genotyping protocol

Volume: 1 L **Storage:** Room temperature indefinitely

Ingredients

Component	Final Concentration	Amount
Tris base	2 M	**242 g**
Glacial acetic acid	1 M	**57.1 mL**
EDTA (0.5 M, pH 8.0)	50 mM	**100 mL**
ddH_2O	-	**to 1 L**

Preparation

1. Add ~700 mL ddH_2O to beaker
2. Add Tris base, stir until dissolved
3. Add glacial acetic acid (CAREFULLY - exothermic)
4. Add EDTA solution
5. Bring to 1 L with ddH_2O
6. Store at room temperature

> **Important Notes**
> Dilute 1:50 for 1X TAE running buffer (20 mL 50X TAE + 980 mL water). Used for agarose gel electrophoresis.

TAE buffer | gel electrophoresis | agarose

Complete Growth Medium (DMEM)

Cell Culture Media

From: Cell Culture protocol

Volume: 500 mL **Storage:** 4°C for 1 month

Ingredients

Component	Final Concentration	Amount
DMEM (high glucose)	-	**445 mL**

Component	Final Concentration	Amount
Fetal Bovine Serum (FBS)	10%	**50 mL**
Penicillin-Streptomycin (100X)	1X	**5 mL**

Preparation

1. In sterile hood, add FBS to DMEM bottle
2. Add Pen-Strep
3. Swirl gently to mix
4. Label with date and contents
5. Store at 4°C

> **Important Notes**
>
> FBS concentration varies by cell line (typically 5-15%). Warm media to 37°C before adding to cells. Some cell lines require additional supplements (L-glutamine, non-essential amino acids, etc.).

cell culture · media · DMEM · FBS

Neurosphere Media (Neural Progenitors)

Cell Culture Media

From: Neural Progenitor Culture protocol

Volume: 50 mL **Storage:** 4°C for 2 weeks

Ingredients

Component	Final Concentration	Amount
Neurobasal Medium	-	**48 mL**
B27 Supplement without Vitamin A (50X)	1X	**1 mL**

Component	Final Concentration	Amount
L-glutamine (200 mM)	2 mM	**0.5 mL**
Penicillin-Streptomycin (100X)	1X	**0.5 mL (1% total volume)**
EGF (stock 20 µg/mL)	20 ng/mL	**Add fresh**
FGF-2 (stock 20 µg/mL)	10 ng/mL	**Add fresh**

Preparation

1. Combine Neurobasal, B27 without Vitamin A, and L-glutamine
2. Store base media at 4°C
3. Add growth factors (EGF and FGF-2) fresh each time
4. Warm to 37°C before use

> **Important Notes**
> EGF and FGF-2 are unstable and must be added fresh. Do not store complete media with growth factors for more than 1 week. B27 without Vitamin A is used to prevent interference with retinoid signaling pathways.

neurosphere · neural progenitors · growth factors · Neurobasal · B27-VitA

Serum Free Media (DMEM/F12-based)

Cell Culture Media

From: Neural Progenitor Culture protocol

Volume: 50 mL **Storage:** 4°C for 2 weeks

Ingredients

Component	Final Concentration	Amount
DMEM/F12 (1:1)	-	**48.5 mL**
B27 Supplement without Vitamin A (50X)	1X	**1 mL**
Penicillin-Streptomycin (100X)	1X	**0.5 mL (1% total volume)**
EGF (stock 20 µg/mL)	20 ng/mL	**Add fresh**
FGF-2 (stock 20 µg/mL)	10 ng/mL	**Add fresh**

Preparation

1. Combine DMEM/F12 and B27 without Vitamin A
2. Store base media at 4°C
3. Add growth factors (EGF and FGF-2) fresh each time
4. Warm to 37°C before use

> **Important Notes**
> EGF and FGF-2 are unstable and must be added fresh. Do not store complete media with growth factors for more than 1 week. DMEM/F12 alternative to Neurobasal for neural progenitor culture. L-glutamine not required as DMEM/F12 formulations often include stable glutamine (GlutaMAX).

serum free | neural progenitors | growth factors | DMEM/F12 | B27-VitA

Astrocyte Culture Medium

Cell Culture Media

From: Astrocyte Isolation protocol

Volume: 500 mL **Storage:** 4°C for 1 month

Ingredients

Component	Final Concentration	Amount
DMEM/F12 (1:1)	-	**445 mL**
Fetal Bovine Serum	10%	**50 mL**
Penicillin-Streptomycin	1X	**5 mL**

Preparation

1. Combine all components in sterile hood
2. Mix well
3. Store at 4°C
4. Warm to 37°C before use

> **Important Notes**
> DMEM/F12 blend provides optimal nutrient balance for glial cells. FBS can be reduced to 5% after initial plating.

astrocytes DMEM/F12 glial cells

Perfusion Buffer 1 (EGTA)

Perfusion Buffers

From: Hepatocyte Isolation protocol

Volume: 500 mL **Storage:** 4°C for 1 week

Ingredients

Component	Final Concentration	Amount
HBSS (Ca^{2+}/Mg^{2+} free)	-	**500 mL**
EGTA	0.5 mM	**95 mg**
HEPES	10 mM	**1.19 g**

Preparation

1. Add EGTA and HEPES to HBSS
2. Adjust pH to 7.4 with NaOH
3. Filter sterilize (0.22 µm)
4. Store at 4°C
5. Warm to 37°C before perfusion

> **Important Notes**
> EGTA chelates Ca^{2+} to disrupt cell-cell junctions. Keep sterile and warm during perfusion.

perfusion EGTA hepatocyte isolation HBSS

Perfusion Buffer 2 (Liberase)

Perfusion Buffers

From: Hepatocyte Isolation protocol

Volume: 50 mL **Storage:** Make fresh

Ingredients

Component	Final Concentration	Amount
HBSS (with Ca^{2+}/Mg^{2+})	-	**50 mL**
Liberase TM	0.2-0.4 mg/mL	**10-20 mg**
HEPES	10 mM	**119 mg**

Preparation

1. Add HEPES to HBSS with Ca^{2+}/Mg^{2+}
2. Adjust pH to 7.4
3. Filter sterilize
4. Add Liberase TM just before use
5. Warm to 37°C

Important Notes

Make fresh each time. Liberase activity varies by lot - optimize concentration. Requires Ca^{2+} for enzyme activity.

perfusion | Liberase | collagenase | digestion

Hematoxylin Solution (Harris)

Staining Solutions

From: H&E Staining protocol

Volume: Purchase ready-made **Storage:** Room temperature

Ingredients

Component	Final Concentration	Amount
Hematoxylin	-	**Commercial solution**

Preparation

1. Purchase Harris Hematoxylin solution
2. Filter before use to remove precipitates
3. Can be reused multiple times

Important Notes

Stains nuclei blue/purple. Commercially available solutions are safer than preparing from scratch (contains aluminum salts and acidic components).

H&E | hematoxylin | nuclear stain | histology

Eosin Y Solution

Staining Solutions

From: H&E Staining protocol

Volume: 500 mL **Storage:** Room temperature indefinitely

Ingredients

Component	Final Concentration	Amount
Eosin Y powder	1% (w/v)	**5 g**
95% Ethanol	-	**500 mL**
Glacial acetic acid	0.5%	**2.5 mL**

Preparation

1. Dissolve Eosin Y in 95% ethanol
2. Add glacial acetic acid
3. Filter if needed
4. Store in dark bottle at room temperature

> **Important Notes**
> Stains cytoplasm and extracellular matrix pink. Acetic acid intensifies staining. Can be reused.

H&E eosin cytoplasmic stain histology

BODIPY 493/503 Stock Solution

Staining Solutions

From: BODIPY Staining protocol

Volume: 1 mL **Storage:** -20°C protected from light

Ingredients

Component	Final Concentration	Amount
BODIPY 493/503 powder	1 mg/mL	**1 mg**

Component	Final Concentration	Amount
DMSO (anhydrous)	-	**1 mL**

Preparation

1. Dissolve BODIPY powder in DMSO
2. Vortex well
3. Aliquot into 50-100 µL portions
4. Wrap in foil to protect from light
5. Store at -20°C

> **Important Notes**
> Light sensitive! Working concentration is typically 1-5 µg/mL (1:200 to 1:1000 dilution). Dilute in PBS or culture media.

BODIPY | lipid droplets | fluorescence | DMSO stock

Citrate Antigen Retrieval Buffer

Staining Solutions

From: Immunohistochemistry protocol

Volume: 1 L **Storage:** Room temperature for 1 month

Ingredients

Component	Final Concentration	Amount
Sodium citrate tribasic	10 mM	**2.94 g**
Tween-20	0.05%	**0.5 mL**
ddH_2O	-	**to 1 L**

Preparation

1. Dissolve sodium citrate in water

2. Adjust pH to 6.0 with citric acid or HCl
3. Add Tween-20
4. Mix well

> **Important Notes**
> Heat slides in this buffer (microwave or pressure cooker) to unmask epitopes. Most common retrieval method for IHC.

IHC | antigen retrieval | citrate buffer | heat-induced

DAB Substrate Solution

Staining Solutions

From: Immunohistochemistry protocol

Volume: 10 mL **Storage:** Make fresh

Ingredients

Component	Final Concentration	Amount
DAB tablets or powder	0.5 mg/mL	**5 mg**
PBS or Tris buffer pH 7.6	-	**10 mL**
Hydrogen peroxide (30%)	0.03%	**10 µL**

Preparation

1. Dissolve DAB in PBS (protect from light)
2. Add H_2O_2 immediately before use
3. Apply to slides within 5 minutes
4. Develops brown color in 1-10 minutes

> **Important Notes**

> CARCINOGEN! Use with extreme caution. Wear double gloves. Prepare in fume hood. Inactivate used DAB with bleach before disposal.

DAB chromogen IHC hazardous

cDNA Synthesis Master Mix (per 20 µL reaction)

PCR & Molecular

From: RNA Isolation and cDNA Synthesis protocol

Volume: 10 µL (per reaction) **Storage:** Make fresh on ice

Ingredients

Component	Final Concentration	Amount
10X RT Buffer	1X final	**2.0 µL**
25X dNTP Mix (100 mM)	4 mM final	**0.8 µL**
10X RT Random Primers	1X final	**2.0 µL**
MultiScribe™ RT Enzyme	-	**1.0 µL**
Nuclease-free H_2O	-	**4.2 µL**
RNA template	-	**10 µL**

Preparation

1. Thaw kit components on ice
2. Calculate number of reactions + 10% extra
3. Prepare master mix (add enzyme LAST)
4. Mix gently by pipetting
5. Aliquot 10 µL per PCR tube
6. Add 10 µL RNA (up to 2 µg) to each tube
7. Run thermal cycler: 25°C/10min, 37°C/120min, 85°C/5min

Important Notes

Keep all reagents on ice. Add enzyme last. Mix for multiple samples to ensure consistency.

cDNA synthesis | reverse transcription | RT | master mix

100mM PMSF Stock

Lysis Buffers

From: Western Blot protocol

Volume: 10 mL **Storage:** -20°C for 6 months (light-sensitive)

Ingredients

Component	Final Concentration	Amount
PMSF powder	100 mM	**174.2 mg**
DMSO (anhydrous)	-	**10 mL**

Preparation

1. Measure PMSF powder and place in 15 mL conical tube
2. Add 10 mL DMSO
3. Vortex thoroughly to dissolve
4. Aliquot 1 mL per tube
5. Wrap tubes in aluminum foil (light-sensitive)
6. Store at -20°C

Important Notes

Use at 1:100 dilution in RIPA buffer (10 µL per 1 mL RIPA). PMSF is a serine protease inhibitor. Unstable in aqueous solutions (half-life ~30 min at pH 7). DMSO stock is more stable.

PMSF protease inhibitor serine protease DMSO

1M DTT Stock

Lysis Buffers

From: Western Blot protocol

Volume: 1 mL **Storage:** -20°C for 1 year (aliquoted)

Ingredients

Component	Final Concentration	Amount
1,4-Dithiothreitol (DTT)	1 M	**154.2 mg**
10mM Sodium Acetate pH 5.2	-	**1 mL**

Preparation

1. Dissolve DTT in 10mM sodium acetate pH 5.2
2. Vortex until completely dissolved
3. Aliquot 50-100 µL per tube
4. Store at -20°C

> **Important Notes**
> Use at 1:1000 dilution in RIPA buffer (1 µL per 1 mL RIPA for 1mM final). DTT is a strong reducing agent that prevents disulfide bond formation. Do NOT refreeze after thawing.

DTT reducing agent disulfide reduction

200mM Sodium Orthovanadate (Activated)

Lysis Buffers

From: Western Blot protocol

Volume: 50 mL **Storage:** -20°C (aliquoted)

Ingredients

Component	Final Concentration	Amount
Sodium orthovanadate (Na_3VO_4)	200 mM	**1.84 g**
Purified water	-	**45 mL**
NaOH or HCl (1 N)	-	**for pH adjustment**

Preparation

1. Dissolve Na_3VO_4 in 45 mL water with stirring
2. Adjust pH to 10 with 1N NaOH or HCl (solution turns yellow)
3. Boil until solution turns colorless (~10 min)
4. Cool to room temperature
5. Readjust pH to 10
6. Repeat boiling/cooling/pH adjustment until solution remains colorless at pH 10
7. Adjust final volume to 50 mL
8. Aliquot 1 mL per tube
9. Store at -20°C

Important Notes

Powerful phosphatase inhibitor. Upon thawing, heat briefly at 90-100°C and vortex to redissolve crystals. Use at various dilutions depending on application.

sodium orthovanadate | phosphatase inhibitor | tyrosine phosphatase

10X APS (Ammonium Persulfate)

Electrophoresis

From: Western Blot protocol

Volume: 10 mL **Storage:** 4°C for 1 month

Ingredients

Component	Final Concentration	Amount
Ammonium persulfate (APS)	10% (w/v)	**1 g**
ddH_2O	-	**10 mL**

Preparation

1. Dissolve 1 g APS in 10 mL water
2. Vortex to mix completely
3. Aliquot 1 mL per tube
4. Label "10X APS" with date
5. Store at 4°C

> **Important Notes**
> APS initiates polymerization of acrylamide gels. Make fresh monthly. Used with TEMED for gel polymerization. Do NOT freeze.

APS | gel polymerization | polyacrylamide | SDS-PAGE

Ponceau S Stain

Transfer & Blocking

From: Western Blot protocol

Volume: 100 mL **Storage:** Room temperature indefinitely (reusable)

Ingredients

Component	Final Concentration	Amount
Ponceau S powder	0.1% (w/v)	**0.1 g**

Component	Final Concentration	Amount
Glacial acetic acid	5% (v/v)	**5 mL**
ddH_2O	-	**to 100 mL**

Preparation

1. Dissolve 0.1 g Ponceau S in ~80 mL water
2. Add 5 mL glacial acetic acid
3. Stir until completely dissolved
4. Filter with Whatman paper if needed
5. Adjust volume to 100 mL
6. Store in designated bottle

Important Notes

Reversible protein stain for membranes. Stains protein bands red/pink. Use to verify transfer efficiency before blocking. Can be reused many times. Destain with TBS-T.

Ponceau S | membrane stain | transfer verification | reversible

Tail Digestion Buffer

PCR & Molecular

From: Mouse Genotyping protocol

Volume: 500 mL **Storage:** Room temperature indefinitely

Ingredients

Component	Final Concentration	Amount
1M Tris-HCl pH 8.0	10 mM	**5 mL**
0.5M EDTA pH 8.0	10 mM	**10 mL**
5M NaCl	50 mM	**5 mL**

Component	Final Concentration	Amount
10% SDS (autoclaved water)	0.5%	**25 mL**
Autoclaved H_2O	-	**455 mL**

Preparation

1. Add all components EXCEPT SDS to 500 mL bottle
2. Autoclave the solution
3. After cooling, add 25 mL 10% SDS
4. Mix by shaking
5. Store at room temperature

> **Important Notes**
> Used for digesting mouse tail clips. Add proteinase K to 100 µg/mL final concentration immediately before use. Digest at 55°C overnight.

tail digestion | genotyping | DNA extraction | proteinase K

10mg/mL Proteinase K Stock

PCR & Molecular

From: Mouse Genotyping protocol

Volume: 5 mL **Storage:** -20°C (aliquoted)

Ingredients

Component	Final Concentration	Amount
Proteinase K powder	10 mg/mL	**50 mg**
1M Tris-HCl pH 7.5	10 mM	**50 µL**
1M $CaCl_2$	20 mM	**100 µL**
50% Glycerol	50%	**4.85 mL**

Preparation

1. Combine Tris-HCl, $CaCl_2$, and glycerol in tube
2. Add proteinase K powder
3. Mix gently until dissolved (do NOT vortex vigorously)
4. Aliquot 500 µL per tube using filtered tips
5. Store at -20°C

> **Important Notes**
> Use at 1:100 dilution in tail digestion buffer (100 µg/mL final). Proteinase K digests proteins to release DNA. Glycerol prevents freezing and allows storage at -20°C.

proteinase K | DNA extraction | protein digestion | genotyping

10X Agarose Gel Sample Buffer (BB + XC)

PCR & Molecular

From: Mouse Genotyping protocol

Volume: 5 mL **Storage:** 4°C indefinitely

Ingredients

Component	Final Concentration	Amount
Bromophenol blue	-	**12.5 mg**
Xylene cyanol	-	**12.5 mg**
1M Tris pH 7.5	-	**247.5 µL**
50% Glycerol	-	**3 mL**
ddH_2O	-	**2 mL**

Preparation

1. Dissolve bromophenol blue and xylene cyanol in water

2. Add Tris pH 7.5
3. Add glycerol
4. Adjust volume with water to 5 mL
5. Mix well
6. Aliquot 1 mL per tube
7. Store at 4°C

> **Important Notes**
> Bromophenol blue runs at ~300 bp, xylene cyanol at ~4000 bp on 1% agarose gel. Use 1 µL loading buffer per 10 µL DNA sample. Glycerol makes sample sink into wells.

loading buffer | bromophenol blue | xylene cyanol | agarose gel

10X PCR Buffer (KCl-based)

PCR & Molecular

From: Mouse Genotyping protocol

Volume: 36 mL **Storage:** -20°C (aliquoted)

Ingredients

Component	Final Concentration	Amount
3M KCl	500 mM	**6 mL**
1M Tris pH 9.0	100 mM	**3.6 mL**
1M $MgCl_2$	25 mM	**0.9 mL**
Autoclaved H_2O	-	**25.5 mL**

Preparation

1. Combine all components
2. Mix thoroughly
3. Filter sterilize with 0.22 µm filter

4. Aliquot 1.2 mL per tube
5. Store at -20°C

> **Important Notes**
> Generic PCR buffer for use with Taq polymerase. Adjust $MgCl_2$ concentration (15-25mM) based on primer requirements. Use at 1:10 dilution in PCR reactions.

PCR buffer | Taq polymerase | KCl | magnesium

EGF Stock (10 µg/mL)

Cell Culture Media

From: Neural Progenitor Culture protocol

Volume: 10 mL **Storage:** -80°C (aliquoted)

Ingredients

Component	Final Concentration	Amount
Mouse EGF (lyophilized)	10 µg/mL	**100 µg vial**
0.5% BSA in PBS (filtered)	-	**10 mL**

Preparation

1. Prepare 0.5% BSA in PBS and filter sterilize
2. Add 10 mL filtered BSA-PBS to EGF vial
3. Mix gently to dissolve
4. Pre-chill aliquot tubes on ice
5. Aliquot 500 µL per tube
6. Label "mEGF 10 µg/mL" with date
7. Store at -80°C

> **Important Notes**

Working concentration: 20 ng/mL (1:500 dilution). Epidermal growth factor promotes neural progenitor proliferation. Thaw on ice, do NOT refreeze.

EGF growth factor neural progenitors neurosphere

hbFGF Stock (10 µg/mL)

Cell Culture Media

From: Neural Progenitor Culture protocol

Volume: 2.5 mL **Storage:** -80°C (aliquoted)

Ingredients

Component	Final Concentration	Amount
Human basic FGF (lyophilized)	10 µg/mL	**25 µg vial**
0.5% BSA in PBS (filtered)	-	**2.5 mL**

Preparation

1. Prepare 0.5% BSA in PBS and filter sterilize
2. Add 2.5 mL filtered BSA-PBS to hbFGF vial
3. Mix gently to dissolve
4. Pre-chill aliquot tubes on ice
5. Aliquot 250 µL per tube
6. Label "hbFGF 10 µg/mL" with date
7. Store at -80°C

Important Notes

Working concentration: 10 ng/mL (1:1000 dilution). Human basic fibroblast growth factor promotes neural progenitor survival and proliferation. Thaw on ice, do NOT refreeze.

FGF bFGF growth factor neural progenitors neurosphere

Polybrene Stock (800 µg/mL)

Cell Culture Media

From: Lentivirus Production protocol

Volume: 20 mL **Storage:** -20°C (aliquoted)

Ingredients

Component	Final Concentration	Amount
Polybrene (Hexadimethrine bromide)	800 µg/mL	**16 mg**
0.9% NaCl (sterile)	-	**20 mL**

Preparation

1. Weigh 16 mg polybrene
2. Dissolve in 20 mL sterile 0.9% NaCl
3. Filter-sterilize using 0.22 µm syringe filter
4. Aliquot 1 mL per tube
5. Store at -20°C

> **Important Notes**
> Use at 1:100 dilution (8 µg/mL final). Polybrene is a cationic polymer that enhances viral transduction by neutralizing charge repulsion between virus and cell membrane.

polybrene lentivirus transduction enhancer infection

Puromycin Stock (1 mg/mL)

Cell Culture Media

From: Lentivirus Production protocol

Volume: 3 mL **Storage:** -20°C (aliquoted)

Ingredients

Component	Final Concentration	Amount
Puromycin dihydrochloride	1 mg/mL	**3 mg**
Sterile H_2O	-	**3 mL**

Preparation

1. Weigh 3 mg puromycin
2. Dissolve in 3 mL sterile water
3. Filter-sterilize using 0.22 µm syringe filter
4. Aliquot 1 mL per tube
5. Store at -20°C

> **Important Notes**
>
> Working concentration: 1-10 µg/mL (1:1000 to 1:100 dilution). Antibiotic for selecting cells with puromycin resistance gene. Determine optimal concentration by kill curve.

puromycin | selection antibiotic | lentivirus | resistance

4% Paraformaldehyde (PFA)

Staining Solutions

From: H&E / IF / IHC protocol

Volume: 300 mL **Storage:** 4°C for 1 week

Ingredients

Component	Final Concentration	Amount
Paraformaldehyde powder	4% (w/v)	**12 g**
1X PBS	-	**300 mL**
1N NaOH	-	**~8-9 µL per 100 mL**

Preparation

1. SAFETY: Wear mask and work in fume hood - PFA is toxic!
2. Heat 250 mL PBS to 60-70°C
3. Add 12 g PFA powder while stirring
4. Stir until solution becomes clear
5. Add PBS to bring volume to 300 mL
6. Add 8-9 µL 1N NaOH per 100 mL to adjust pH
7. Filter with Whatman paper using funnel
8. Store at 4°C

> **Important Notes**
>
> HAZARD: PFA is toxic and carcinogenic! Always work in fume hood. Use for tissue/cell fixation. Do NOT autoclave (will polymerize). Make fresh weekly for best results.

PFA paraformaldehyde fixative histology hazardous

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