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REAGENTS AND BUFFERS

Phosphate-Buffered Saline (PBS) (10X)

80g of NaCl
2.0g of KCl
14.4g of Na2HPO4
2.4g of KH2PO4
Mix 800mL ultra-pure water and adjust pH to 7.6 with pure HCl. Top up with ultra-pure water to 1L.

Phosphate-Buffered Saline w/ Tween20 (PBST)

For 1L: 100mL of PBS 10x + 890mL ultra-pure water + 10 mL Tween 20

Tris-Buffered Saline (TBS) (10X)

24.23g Trizma HCl
80.06g NaCl
Mix in 800mL ultra-pure water and adjust pH to 7.6 with pure HCl. Top up with ultra-pure water to 1L.

Tris-Buffered Saline w/ Tween20 (TBST)

For 1L: 100mL of TBS 10x + 890mL ultra-pure water + 10 mL Tween 20

WESTERN BLOTTING BUFFERS

RIPA Buffer

150mM NaCl
1.0% NP-40 or 0.1% Triton X-100
0.5% sodium deoxycholate
0.1% SDS (sodium dodecyl sulphate)
50mM Tris-HCl pH8.0
Protease Inhibitors

Tris-HCl buffer

20mM Tris-HCl pH7.5
Protease Inhibitors

Denaturing lysis buffer

1% SDS
5mM EDTA
Immediately before use add:
10mM dithiothreitol or beta-mercaptoethanol
Protease inhibitors
15U/mL DNase1

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Laemmli 2X buffer/loading buffer

- 4%SDS
- 10% 2-mercaptoethanol
- 20% glycerol
- 0.004%bromophenol blue
- 0.125M Tris-HCl
- Check the pH and adjust pH to 6.8.

Running buffer

- 25mM Tris base
- 190mM glycine
- 0.1% SDS
- Check the pH, which should be about pH 8.3. Adjust if necessary.

Transfer buffer (wet)

- 25mM Tris base
- 190mM glycine
- 0.1% SDS
- The pH should be about pH8.3. Adjust if necessary.

Transfer buffer (semi-dry)

- 48mM Tris
- 39mM glycine
- 20% methanol
- 0.04% SDS

Blocking buffer

- 5% milk or BSA(bovine serum albumin)
- Add to TBST buffer. Mix well and filter.

IMMUNOHISTOCHEMISTRY/IMMUNOCYTOCHEMISTRY BUFFERS

Formalin solution (10%)

- 3.7-4% Formaldehyde (37-40%)
- 33mM NaH_2PO_4
- 46mM Na_2HPO_4

Paraformaldehyde (4%)

- 8% paraformaldehyde

0.2M Phosphate Buffer(PB) pH7.4

- 53mM NaH_2PO_4
- 154mM Na_2HPO_4

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Heat 8%PFA solution at 60C while stirring. Once the solution reaches 60C and the PFA dissolves, add 500mL of 0.2M phosphate buffer, to bring the solution to 4%PFA in 0.1M phosphate. Carefully add 1N NaOH until the solution is clear. Cool the solution and filter.

Sodium Citrate Buffer pH6.0

10mM sodium citrate
0.05% Tween20
Mix to dissolve sodium citrate and adjust pH to 6.0 with 1N HCl.
Add Tween20 and mix well.
Store at room temperature for 3 months or at 4C for longer storage.

ELISA BUFFERS

Bicarbonate/carbonate coating buffer (1oomM) pH9.6

Antigen or antibody should be diluted in coating buffer to immobilize them to the wells:
29mM Na2CO3
71mM NaHCO3

Blocking solution

Commonly used blocking agents are 1% BSA, serum, or non-fat dry milk in PBS.

Wash solution

Usually PBS or Tris-buffered saline(pH7.4) with detergent such as 0.05% (v/v) Tween 20 (TBST).

FLOW CYTOMETRY

FACS buffer/antibody dilution buffer

10% FCS
1% sodium azide in PBS

Permeabilization

0.1-1% Triton X-100/NP-40 in PBS

Fixative

0.01-0.1% paraformaldehyde in PBS.