mAb3D Buffer Recipes

***Contents*:**

I. Suggested Reagents

Reagent	*Reference*
Agarose	Invitrogen 16500500
Dichloromethane (DCM)	Sigma 270997-12X100ML
Glycine	Sigma G7126-500G
H_2O	MilliQ double distilled water
Heparin	Sigma H3393-50KU
Iohexol	Nycodenz®, Axis-Shield, Cosmo Bio USA AXS-1002424 or Histodenz™, Sigma D2158-100G
Methanol (MeOH)	Fisher A412SK-4
Paraformaldehyde 16% (PFA)	EMS 15710-S
Phosphate buffered saline (PBS) 10X	Ambion AM9624, or prepare solution per below recipe
Potassium chloride (KCl)	Sigma P5405-250G
Potassium phosphate monobasic (KH_2PO_4)	Fisher P285-500
Sodium azide (NaN_3)	Sigma 58032-100G
Sodium chloride (NaCl)	Fisher S640-3
10N Sodium hydroxide (NaOH)	Fisher SS255-1

Sodium phosphate dibasic dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$)	Sigma 71650
Sodium phosphate monobasic dihydate ($NaH_2PO_4 \cdot 2H_2O$)	Sigma 71500
2,2′-Thiodiethanol (TDE)	Sigma 166782-500G
Triton X-100	Sigma X100-500ML
Tween-20	Sigma P9416-100ML

II. Buffer Recipes

Sample Collection

20mg/mL Heparin (stock): 14 mL

- Heparin salt……280 mg
- distilled H_2O……...14 mL

Add 14 mL DI H_2O to 15 mL conical tube. Weigh, add heparin. Centrifuge.

10x PBS w/ heparin (stock): 1 L

- $Na_2HPO_4 \cdot 12H_2O$……..34.2 g
- NaCl……...80.0 g
- KCl……2.0 g
- KH_2PO_4 ……...2.0 g
- 20mg/mL Heparin……..5.0 mL
- distilled H_2O

Bring to 1 L with DI H_2O. Mix at RT until fully dissolved, filter; **store at RT**. To use for perfusion, dilute by factor of ten to yield 1xPBS w/ 10µg/mL heparin. Prepare buffer without heparin for tissue storage media.

4% PFA: 200 mL

- 10x PBS w/ heparin…….20 mL
- PFA 16% .....…… 20 mL
- distilled H_2O……130 uL

Prepare fresh each time; **keep on ice** when not in use.

B1n: 500 mL

- distilled H_2O…….500 mL
- Glycine……10 g
- Triton X-100……..500 uL
- NaOH, 10N……..50 uL
- 20% NaN_3……..200 uL

Add water first with stir bar to prevent clumping. Add components while stirring gently. Triton is thick and sticky, need to pipette up and down; can drop pipette tip into flask to allow rest to diffuse out. Mix at RT until fully dissolved (~1hr); **store at RT**.

Delipidation

***Stock solutions*:**

10x PBS: 1 L

- $Na_2HPO_4 \cdot 12H_2O$……..34.2 g
- NaCl……..80.0 g
- KCl……2.0 g
- KH_2PO_4 ……2.0 g
- distilled H_2O

Bring to 1 L with DI H_2O. Mix at RT until fully dissolved; **keep at RT**.

1x PBS w/0.02% NaN_3: 1 L

- 10x PBS……..100 mL
- 20% NaN_3 ……...1 mL
- distilled H_2O

Fill with distilled water to 1L. Mix at RT; **keep at RT**.

***Solutions for Delipidation*:**

MeOH gradients: 50 mL

	20%	40%	60%	80%
MeOH	10 mL	20 mL	30 mL	40mL
B1n	40 mL	30 mL	20 mL	10 mL

Mix and cool down before use; **keep at RT**.
Note: MeOH/B1n series may have salt precipitation at high concentration. Use the top solution.

PTxwH: 1L

- distilled H_2O…….900 mL
- 10x PBS……100 mL
- Triton X-100……..1 mL
- Tween-20……...500 uL
- Heparin (20mg/ml)…….... 100 uL
- 20% NaN_3 …….1 mL

Mix at RT until fully dissolved; **keep at RT**.

Section-based Antibody Screening

1x PBS w/0.2% NaN_3 (high concentration): 50 mL

- 10x PBS……5.0 mL
- 20% NaN_3 ……0.5 mL
- distilled H_2O……...45.0 mL

Mix at RT; **keep at RT**. **Use**: to initially fill well plates for sections.

20 mM PB: 1L

- $Na_2HPO_4 \cdot 12H_2O$…(sodium phosphate dibasic dodecahydrate)……...5.73 g
- $NaH_2PO_4 \cdot 2H_2O$…...(sodium phosphate monobasic dihydate)……..0.624 g
- distilled H_2O

Bring to 1 L with DI H_2O. Mix at RT until fully dissolved; **keep at RT**.

ACB high RI mounting media: in 50ml conical tube

- 20 mM PB……8.0 mL
- 2,2′-Thiodiethanol (TDE)……....22.4 mL
- Iohexol (Histodenz or Nycodenz)……24.0 g

Mix the PB buffer and TDE first to prevent clumping. Shake at RT, 200 rpm o/n, until dissolved. Spin down at 5,000 rpm for 10 min to remove any precipitate. Aliquot for use at **RT**.