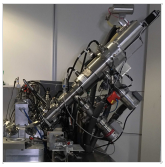

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MIBI and IHC solutions

Forked from IHC staining

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1 Works for me dx.doi.org/10.17504/protocols.io.bhmej43e

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ABSTRACT

These protocols are designed to prepare solutions to be used for the Multiplex Ion Beam Imaging Time of Flight instrument (MIBI_TOF) and IHC related protocols in the Bendall and Angelo labs. Particularly, the IHC protocol uses the same reagents and steps used for MIBI staining. Therefore, It can be used to validate the compatibility of a new target antibody before use for MIBI staining. These protocols have been successfully use for MIBI and are the result of extensive optimization experiments.

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FORK NOTE

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GUIDELINES

Use sterile single use 50 mL tube for measurement

Use the measurement marks on the Nalgene bottle to complete with water

Do not use any other vessels then single use graduated plastic containers to prevent cross contamination

MATERIALS TEXT

A	B	C
Products	**Provider**	**Catalogue No.**
TBS IHC Wash Buffer with Tween 20	Cell Marque	935B-09
PBS IHC Wash Buffer with Tween 20	Cell Marque	934B-09
Target Retrieval Solution, pH 9, (3:1)	Agilent (Dako)	S2375
Tris Base, BioReagents	Fisher Scientific	BP154-1
UltraPure water	Invitrogen	10977-015
Ultrapure (type 1, >18 MOhms) water	In house	NA
Hydrochloric acid	Sigma-Aldrich	H1758-100ML
Gelatin (cold water fish skin)	Sigma-Aldrich	G7765-250
Bovine Albumin (BSA), heat shock treated	Fisher Scientific	BP1600-100
Horse serum	Vector Labs	S-2000
VectaMount Permanent Mounting Medium	Vector Labs	H-5000
Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units, 150 mL	Fisher Scientific	09-740-28E
Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units, 500 mL	Fisher Scientific	09-740-28C
Nalgene, bottle 1L	Fisher Scientific	03-312-5
Millex-HV (0.45µm)	Millipore	SLHV033RS
BD Syringes without Needle, 50 mL	Fisher Scientific	13-689-8
Brady label White polyester/polypropylene	Brady	M21-500-7425
BMP21-LAB Printer Kit	Brady	BMP21-LAB

SAFETY WARNINGS

All organic solvents should be manipulated under a chemical hood

BEFORE STARTING

Verify the stocks of all reagents prior starting an experiment

If some reagents are running low, ask to place an order or prepare solutions

Target retrieval solution (Antigen retrieval)

1 Make a fresh 1:10 dilution

2.5 mL of target retrieval solution 10x (3-in-1) pH 9 (Dako)

in **22.5 mL** of ultrapure (type 1, >18 MOhms) water

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Total volume (mL)	Volume (mL) Target retrieval	Volume (mL) H2O
25	2.5	22.5
50	5	45
100	10	90

MIBI or IHC 1X PBS wash buffer

2 Verify stock of **MIBI or IHC 1x PBS wash buffer** and prepare accordingly if running low

2.1 Use the same 1 L Nalgene Bottle, if before expiring date. Replace by a new 1 L Nalgene bottle on monthly basis

Use single use 50 mL tube for measurement

Use the measurement marks on the bottle to complete with water

Do not use any other vessels than single use graduated plastic containers to prevent cross contamination

2.2 **Label name** (Brady label 0.5", M21-500-7425, Font 14 pts bold, format: Banner landscape)

First line: **MIBI or IHC 1X PBS wash buffer**

Second line: **Exp.: MM/DD/YY (**Expiring date, 1 month from the present date)

2.3 Prepare 1x PBS wash buffer as follow:

Add reagent in row 2 and row 3 first, mix gently

than complete with row 4, ultrapure (type 1, >18 MOhms) water

A	B
Reagents	**Qty for 1000 mL**
PBS IHC Wash Buffer with Tween 20 (mL)	50
Bovine Albumin (BSA), heat shock treated (g)	1
Ultrapure (type 1, >18 MOhms) water (mL)	949

2.4 Store at 4˚C

Reuse the same Nalgene bottle to make new 1x PBS wash buffer until the initial expiring date (<1 month)

Antibody Diluent

3 Prepare Antibody Diluent (NHS 3%)

3.1 Prepare antibody diluent preparation buffer (1xTBS-Tween)

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Add **100 mL** UltraPure Water (Invitrogen, 10977-015) in a Nalgene filter unit (Fisher Scientific, 09-740-28E)

3.2 Add the stock solution **7.5 mL** of TBS IHC Wash Buffer with Tween 20 (Cell Marque, 20x)

3.3 Complete to **150 mL** with UltraPure Water (Invitrogen, 10977-015)

3.4 Filter under vacuum

3.5 Label as Antibody diluent preparation buffer (1xTBS-Tween)

3.6 Make a 3% (volume/volume) of normal Horse serum

Add **1.5 mL** **Normal Horse Serum (Vector labs, S-2000)** in **48.5 mL** of Antibody diluent preparation buffer (1xTBS-Tween) in 50 mL tube

3.7 FIlter with a 50 mL syringe (Fisher Scientific, 13-689-8) and filter (Millipore, Millex-HV, SLHV033RS) into a new 50 tube

3.8 Make some small aliquots by distributing 1 mL with a 10 mL serological pipette in 1.5 or 2 mL tubes and stock aliquots of 10 mL in 15 mL tubes

3.9 Label cap tubes using dot printed labels **Antibody Diluent (NHS 3%)**

3.10 Store at -20˚C small aliquots in the staining box reagents and stock aliquots in the specified location

Gelatin 10% stock, 100x

4 Prepare stock 10% weight/ volume cold fish water gelatin

4.1 Warm up the gelatin stock bottle at **37 °C** prior use to reduce viscosity

Put a 15 mL tube on a precision balance and tare it to zero

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4.2

4.3 Take gelatin by aspirating ~ **1 mL** using a large opening pipette tip >4 mm OD

4.4 Add slowly the viscous solution to the tube until it weigh **1 g**

4.5 Warm up **20 mL** Ultrapure Water (Invitrogen, 10977-015) in a 50 mL

4.6 Take **8 mL** of warm Ultrapure Water (Invitrogen, 10977-015) and add into the gelatin 15 mL

4.7 Make sure that all the gelatin is dissolved and complete the volume to **10 mL**

4.8 Make small aliquots by distributing ~1 mL with a 10 mL serological pipette in 1.5 or 2 mL tubes

4.9 Store at -20˚C small aliquots in the staining box reagents

Triton 10% stock, 100x

5 Prepare Triton stock 10% volume/ volume

5.1 Add **9 mL** of Ultrapure Water (Invitrogen, 10977-015) to a **50 mL** tube

5.2 Take Triton by aspirating slowly **1 mL** using a large opening pipette tip >4 mm OD

5.3 Transfer into the **50 mL** tube

5.4 Make sure that all the Triton is dissolved

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5.5 Make small aliquots by distributing ~1 mL with a 10 mL serological pipette in 1.5 or 2 mL tubes

5.6 Store at -20˚C small aliquots in the staining box reagents

Sodium azide (20 mg/mL), 100x

6 Prepare 100x (2%) of sodium azide

6.1 Add **48 mL** of Ultrapure Water (Invitrogen, 10977-015) in a **50 mL**

6.2 Weigh **1 g** of sodium azide

6.3 Add sodium azide in the **50 mL** tube

6.4 Make sure that all the sodium azide is dissolved and complete the volume to **50 mL**

6.5 Make small aliquots by distributing ~1 mL with a 10 mL serological pipette in 1.5 or 2 mL tubes

6.6 Store at 4˚C small aliquots in the reagents box

Blocking Buffer

7 Prepare the blocking buffer following the table:

A	B	C	D
Reagents	**Qty for 10 mL**	**Qty for 30 mL**	**Qty for 50 mL**
1xTBS IHC Wash Buffer with Tween 20 (mL)	9.5	28.5	47.5
Triton 10% (mL)	0.1	0.3	0.5
Gelatin 10% (mL)	0.1	0.3	0.5
Horse serum (mL)	0.2	0.6	1
Sodium azide 20 mg/mL (mL)	0.1	0.3	0.5

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7.1 Filter the blocking buffer with a filter (Millipore, Millex-HV, SLHV033RS) into a new tube

7.2 Make small aliquots by distributing ~1 mL with a 10 mL serological pipette in 1.5 or 2 mL tubes

7.3 Store at -20˚C small aliquots in the staining box reagents

PBS 10x low barium

8 Use a new 1 L Nalgene bottle

9 Add 900 mL of ultrapure (type 1, >18 MOhms) water in 1 L Nalgene bottle

Use the measurement marks on the bottle

Do not use any other vessel to measure the water

10 Weight the following chemicals:

For 1 L add:

- **80 g** NaCl
- **2 g** KCl
- **26.8 g** Na2HPO4.7H_2O
- **2.4 g** KH2PO4

Make sure that the chemical used correspond to the formula, particularly, for the sodium phosphate dibasic heptahydrate

11 Mix thoroughly. Do not adjust pH

12 Filter using 2 x 500 mL vacuum filter unit (Fisher Scientific, 09-740-28C)

13 Label each bottles with Brady lab printer (font 14, bold, banner landscape, tape 1/2") as:

First line: **PBS low barium 10x stock**

Second line: **Date (MM/DD/YY)**

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Tris Buffer pH 8.5 100mM

14 Use a new 1 L Nalgene bottle, label with Brady lab printer (font 14pt, bold, banner landscape, 1/2" tape) as follow:

First line: **100 mM TRIS BUFFER pH8.5**

Second line: **Exp.: MM/DD/YY** (expiration date: add 1 month to the preparation date in a new bottle)

15 Add 900 mL of ultrapure (type 1, >18 MOhms) water in 1 L Nalgene bottle

Use the measurement marks on the bottle

Do not use any other vessel to measure the water

16 Add 12.114 g of Tris Base

17 Mix thoroughly

18 Adjust to 1000 mL with ultrapure (type 1, >18 MOhms) water

19 Add 3.0 mL of Hydrochloric acid (Sigma-Aldrich, H1758-100ML) and mix well

20 Calibrate pH meter

20.1 Press Calibrate on the pH meter

20.2 Wash the pH probe with ultrapure (type 1, >18 MOhms) water, wipe, submerge in buffer 1 (pH 10) and Press Read

20.3 Wait until it stabilizes and says second buffer

20.4 Wash the pH probe with ultrapure (type 1, >18 MOhms) water, wipe, submerge in buffer 2 (pH 7) and Press Read

20.5 If the calibration is successful, the screen should indicate CALIBRATED

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21 Adjust to pH 8.5

21.1 Wash the pH probe with ultrapure (type 1, >18 MOhms), wipe, submerge in the Tris solution

21.2 Press read and wait for the reading to stabilize

21.3 Acceptable pH values range between 8.4 to 8.6, if pH value is in that range, the solution is ready

21.4 If the pH is above 8.6 add 100 µL of HCl

21.5 If HCl has been added measure the pH again **go to step #21.1**

Reuse the same Nalgene bottle to make new Tris Buffer pH 8.5 100mM until the initial expiring date (<1 month)

PBS low barium 1x

22 Use a new 1 L Nalgene bottle, label with Brady lab printer (font 14pt, bold, banner landscape, 1/2" tape) as follow:

First line: **PBS low barium 1x**

Second line: **Exp.: MM/DD/YY** (expiration date: add 1 month to the preparation date in a new bottle)

23 Add 900 mL of ultrapure (type 1, >18 MOhms) water in 1 L Nalgene bottle

Use the measurement marks on the bottle

Do not use any other vessel to measure the water

24 Use 50 mL disposable Falcon tube to measure **100 mL** (2x50 mL) of PBS 10x Low Barium stock solution

25 Mix thoroughly

26 Reuse the same Nalgene bottle to make new PBS low Barium until the initial expiring date (<1 month)

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