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Lyophilized metal-antibody reconstitution

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1 Works for me dx.doi.org/10.17504/protocols.io.bjd6ki9e

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ABSTRACT

This protocol describes the procedure to prepare the reconstitution buffer and the resuspension of metal-labeled antibody.

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MATERIALS TEXT

A	B	C
Material	Provider	Cat. Number
Tris 1M, pH 7.5	ThermoFisher	15567027
Ultrapure Water	ThermoFisher	10977023
Sodium azide (100x, 20 mg/mL)	Sigma Aldrich	S2002-25G
Antibody stabilizer Tris-based	Boca Scientific	130 050
Tube conical super clear, 500µL	VWR	16466-050
Millex-HV (0.45 µm)	Millipore	SLHV033R5

Stock solutions preparation

1 Prepare Tris (10 mM), sodium azide (0.02%)

- 1.1 Add **500 µl** Tris 1M, pH 7.5 in **50 mL** tube
- 1.2 Add **500 µl** sodium azide (**20 mg/ml**)
- 1.3 Complete with 50 mL ultrapure water
- 1.4 Make aliquots of **10 mL** and store at **-20 °C**

2 Prepare reconstitution buffer

A	B	C	D
Reagents	For 10 mL	For 20 mL	For 50 mL
Antibody stabilizer (Tris-based)	3.75	7.5	18.75
Tris (10 mM, pH 7.5), sodium azide (20mg/mL)	6.25	12.5	31.25

- 2.1 **For 10 mL**
Add **3.75 mL** of Antibody stabilizer (**Tris-based**)
- 2.2 Add **6.25 mL** Tris (10 mM), sodium azide
- 2.3 Filter using Millex-HV (0.45 µm) and syringe in new
- 2.4 Make aliquots of **1 mL** and store at **-20 °C**

Antibody suspension

45m

- 3 Take a **5 µg** aliquoted antibody tube and bring at **Room temperature**
 - 3.1 **Carefully open** the screw cap tube

3.2 Add gradually 100 μ L of reconstitution buffer over the lyophilized cake without touching it

3.3 Mix gently by flicking the bottom of the tube

3.4  

30m

Let it sit for at least  **00:30:00** at room temperature to allow rehydration

Highly recommended: store at  **4 °C** overnight to allow longer time for rehydration

3.5 Mix gently by flicking the bottom of the tube, while flicking inspect for translucent pellet and make sure that the lyophilized cake is totally suspended

3.6  **10000 rcf, Room temperature , 00:01:00**

Reconstitution label date

4 Place the suspension dot label at bottom of the tube with the date recorded as MM/DD/YY; 50 μ g/mL



4.1 Store at 4°C or use an aliquot immediately