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Metal-antibody MIBItag conjugation kit

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1 Works for me dx.doi.org/10.17504/protocols.io.bhyej7te

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ABSTRACT

This is a "protocol IO" extended version of MIBItag conjugation kit protocol from Ionpath.

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PROTOCOL CITATION

Marc MB Bosse, Sean Bendall, Mike Angelo 2021. Metal-antibody MIBItag conjugation kit. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bhyej7te

KEYWORDS

null, Ionpath, MIBItag, conjugation, metal, antibody

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38630

MATERIALS TEXT

MATERIALS

Centrifugal Filter Unit: 50 kDa Amicon Ultra 500 µL V

bottom **Millipore Catalog #UFC505096** In 2 steps

Worksheet antibody information

1

A	B	C	D	E	F	G	H	I	J
Antibody (clone)	Isotope/Element	Pre-label Nanodrop (µg/mL)	Volume to label 200 µg	Post-label Nanodrop (µg/mL)	Labelled volume	Dilution for 200 µg/mL	Total volume	Volume of stabilizer	Conjug ID
ALK									

MIBItag conjugation

kit **IonPath Catalog #600XXX**

Optional antibody concentration 10m

10m

2 ⚠

Critical: the 50-kDa MWCO micro-filter device is designed to hold up to 500 µL

If 100 µg of antibody requires addition of more than 200 µL in volume

Add up to **500 µl** of antibody solution in the 50-kDa MWCO micro-filter device

Centrifuge **12000 rcf, Room temperature** **00:10:00** or **21000 rcf, Room temperature , 00:05:00**

Centrifugal Filter Unit: 50 kDa Amicon Ultra 500 µL V

bottom **Millipore Catalog #UFC505096**

2.1 ⚠

Make sure to align the filters parallel to centrifuge force to ensure optimal tengential flow filtration of both filters

This can be done by centering the hinge of the collecting tube between the two filters

The 50-kDa MWCO micro-filter device is then placed in the centrifuge with the hinge outward

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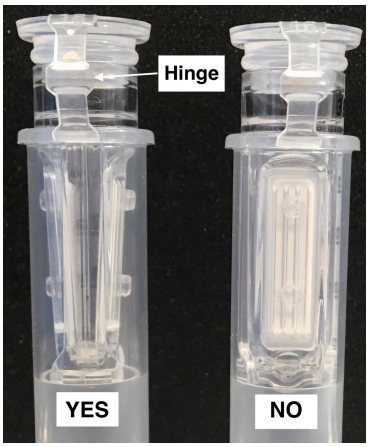

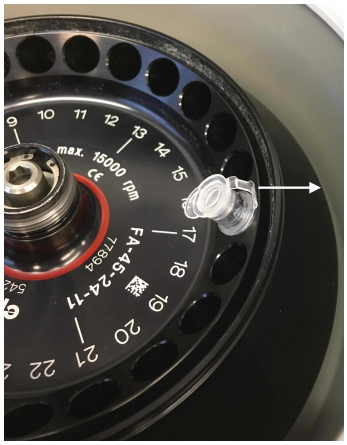

Antibody buffer exchange

3 Add 300 µL of Buffer 2 (100 mM phosphate buffer, 2.5 mM EDTA, pH 7.2) to a 50-kDa MWCO micro-filter device

Centrifugal Filter Unit: 50 kDa Amicon Ultra 500 µL V bottom **Millipore Catalog #UFC505096**

4 If step 2 not performed and antibody solution volume is less than **200 µl**

Add the 100 µg antibody solution volume

5 **12000 rcf, Room temperature** **00:10:00** or **21000 rcf, Room temperature , 00:05:00**

6 Add 400 µL of buffer 2 IonPath

7 ⚠

12000 x g, Room temperature **00:10:00** or **21000 rcf, Room temperature , 00:05:00**

Make sure that the residual dead volume is below the bottom end of the filter (~20 µL)

Do not measure with a pipette, just inspect visually if the level of the fluid is as shown in the pictures

(blue dye was used for demonstration only to help visualizing the fluid)

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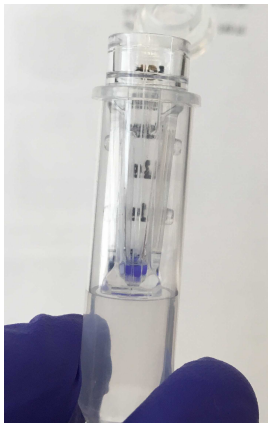

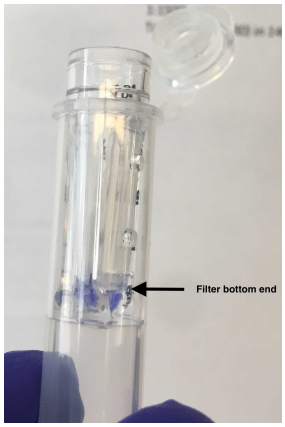

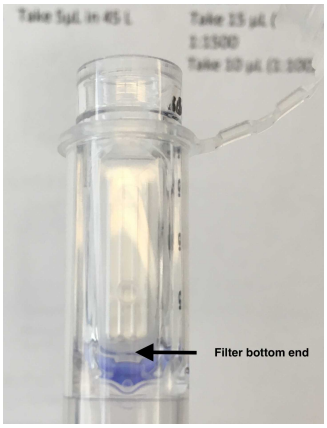

8 Dilute **8 µl** of TCEP (0.5M) in **992 µl** of buffer 2 (**4 Milimolar (mM)** final)

Tris(2-carboxyethyl)phosphine hydrochloride solution **Sigma Aldrich Catalog #646547-10X1ML**

Note: **10 µl** TCEP is aliquots are stored at **-20 °C**

Antibody reduction

9 Add **100 µl** of diluted TCEP to the concentrated antibody in the 50-kDa MWCO micro-filter device

10 Mix antibody and TCEP solution by flicking the 50-kDa MWCO micro-filter device gently

11 ⚠

Incubate at **37 °C** for **00:30:00 ONLY**

Do not incubate longer than 30 min, this will result in full reduction of disulfide bonds necessary for the structural integrity of the antibody

Antibody wash

12 After incubation, add immediately **300 µl** of buffer 3 to the partially reduced antibody in the50-kDa MWCO micro-filter device

13 Mix reduced antibody and wash buffer 3 (Tris buffed salt, 1 mM EDTA, pH 7.5) by flicking the 50-kDa MWCO micro-filter device gently

14 **12000 rcf, Room temperature , 00:10:00** or **21000 rcf, Room temperature , 00:05:00**

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15 Discard the flow through

16 Add additional **400 µl** of buffer 3 **go to step #14 once**

Conjugation of metal and antibody

17 Reconstitute preloaded polymer in **100 µl** of buffer 3

18 Transfer the preloaded polymer solution in the reduced antibody/ 50-kDa MWCO micro-filter device

19 Mix reduced antibody and preloaded polymer by flicking the 50-kDa MWCO micro-filter device gently

20 Inccubate **37 °C** for **01:30:00** to **02:00:00**

Final wash

21 Add 250 µL of buffer 4 to the antibody conjugation mixture in the the 50-kDa MWCO micro-filter device

22 Mix antibody conjugation mixture and buffer 4 (Tris buffed salt, pH 7.5) by flicking the 50-kDa MWCO micro-filter device gently

23 **12000 rcf, Room temperature , 00:10:00** or **21000 rcf, Room temperature , 00:05:00**

24 Discard the flow through

25 Add **400 µl** of buffer 4 in the the 50-kDa MWCO micro-filter device **go to step #22** to 24

26 Add **400 µl** of buffer 4 in the the 50-kDa MWCO micro-filter device **go to step #22** to 24

27 Add **400 µl** of buffer 4 in the the 50-kDa MWCO micro-filter device **go to step #22** to 24

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28 Add **400 µl** of buffer 4 in the the 50-kDa MWCO micro-filter device **go to step #22** to 24

Labelled antibody retrieval

29 Add **50 µl** of buffer 4 to the washed labelled antibody in the the 50-kDa MWCO micro-filter device

30 Mix washed labelled antibody and buffer 4 by flicking the 50-kDa MWCO micro-filter device gently

31 Invert the micro-filter into a new collection tube

32 **10000 rcf, Room temperature , 00:01:00**

33 Add **80 µl** of buffer 4 to the washed labelled antibody in the the 50-kDa MWCO micro-filter device
go to step #30 to 33

34 Measure the volume retrieved with a pipette (200 µL) and register data in labelled volume on the worksheet
go to step #1

35 Open Nanodrop using Protein 280 and chose type = IgG

35.1 Clean the cell with ultrapure (type 1) water

35.2 Blank with buffer 4

35.3 Take **1.5 µl** of labelled antibody and measure concentration

35.4 Register data in labelled volume on the worksheet **go to step #1**

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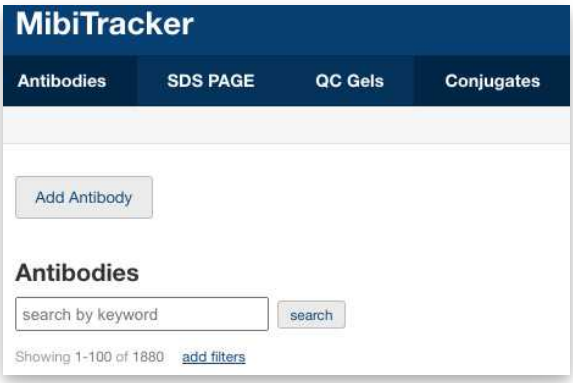
MIBItracker database/storage formulation

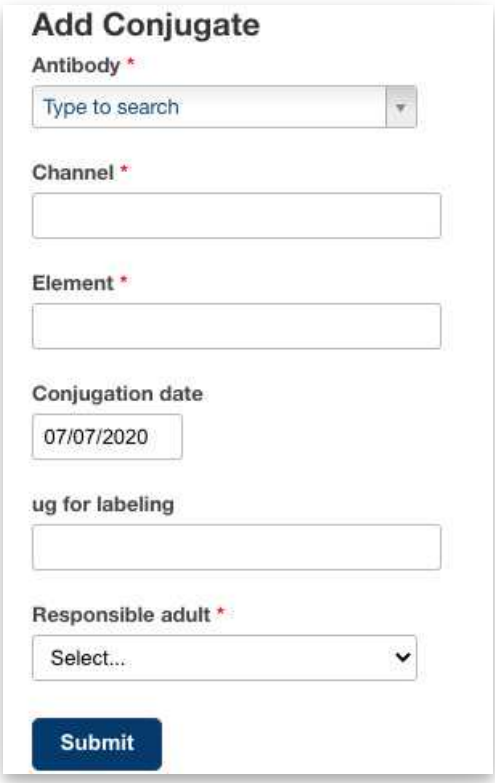
36 Open **MIBItracker** and click on **CONJUGATES** tab

36.1 Click on **Add Conjugate** tab



36.2 Select the antibody reference used for the conjugation

Fill the following fields: **Isotope**/ **Channel**; **Element**; **µg for labelling**; **Responsible**



36.3 Click on **SUBMIT**, this will generate a conjugation ID number

Click on back to **Conjugate** and filter by conjugation date

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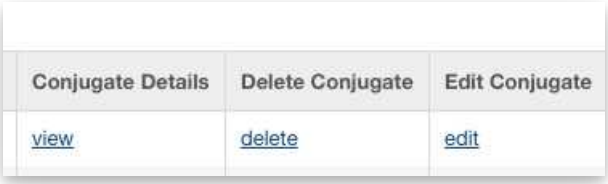
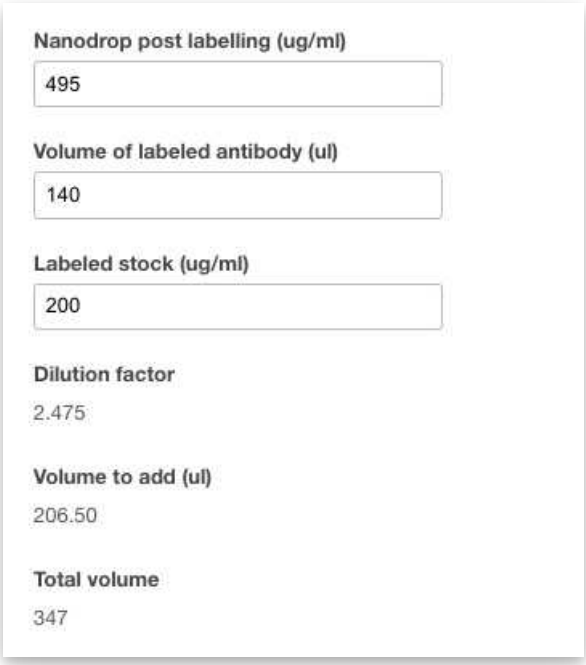
36.4 Record the ID number on your experiment worksheet go to step #1

36.5 Click on Edit Conjugate



36.6 Enter Nanodrop reading post-labelling and Volume of labeled antibody in the respective fields



36.7 For a standard labeled stock concentration (200 µg/mL), the Dilution factor and the volume of Antibody Stabilizer is calculated automatically

If the Dilution factor is less than <1, change labeled stock concentration to 100 µg/mL

36.8 Add the volume of Antibody Stabilizer to the antibody labeled collection tube. Use Tris-based antibody stabilizer (Boca Scientific).

36.9 Transfer the diluted labeled antibody into a new screw cap microcentrifuge tube

36.10 Label the tube with printer Brady 21-Lab, (Label for 1.5 mL vial, Auto bold)

Line1: Target name, Channel

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Line2: ID: number
Line3: date (mm/dd/yy)

36.11 Hand write the target name and the channel on a dot label and place on the screw cap

Short term storage at 4˚C/ Long term storage Lyophilized

37 Labeled can be stored short term for <3 months at 4˚C in solution

Preferably, the newly labeled labeled antibody is immediately placed in the lyophilization box at 4˚C

in queue to be lyophilized and then placed at 4˚C in designated box for longterm storage management

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