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 We use this protocol and it's working

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🌐 Metal-antibody MIBItag conjugation kit

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ABSTRACT

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

MATERIALS

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

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

MIBtag conjugation kit **IonPath Catalog #600XXX**

Optional antibody concentration

10m

2

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

10m

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 **Merck Millipore (EMD Millipore) Catalog #UFC505096**

2.1

Make sure to align the filters parallel to centrifuge force to ensure optimal tangential 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

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 **Merck Millipore (EMD 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 μ L)

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)

8 Dilute 8 μ L of TCEP (0.5M) in 992 μ L of buffer 2 ([M] 4 millimolar (mM) final)

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

Note: 10 μ L TCEP is aliquots are stored at -20 $^{\circ}$ C

Antibody reduction

9 Add 100 μ L 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 the 50-kDa MWCO micro-filter device

13 Mix reduced antibody and wash buffer 3 (Tris buffered 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

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 Incubate 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
- 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](#)

MIBItracker database/storage formulation

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

36.1 Click on **Add Conjugate** tab

The screenshot shows the MibiTracker web application. At the top is a dark blue header with the title "MibiTracker" in white. Below the header is a navigation bar with four tabs: "Antibodies", "SDS PAGE", "QC Gels", and "Conjugates". The "Antibodies" tab is currently selected. Below the navigation bar, there is a light gray box containing a button labeled "Add Antibody". Below this box, the heading "Antibodies" is displayed. Under the heading is a search bar with the placeholder text "search by keyword" and a "search" button. At the bottom of the search area, it says "Showing 1-100 of 1880" followed by a link to "add filters".

36.2 Select the antibody reference used for the conjugation

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

The screenshot shows the "Add Conjugate" form. The title "Add Conjugate" is at the top. Below it are several fields:

- Antibody ***: A dropdown menu with the placeholder text "Type to search".
- Channel ***: A text input field.
- Element ***: A text input field.
- Conjugation date**: A date input field showing "07/07/2020".
- μ g for labeling**: A text input field.
- Responsible adult ***: A dropdown menu with the placeholder text "Select...".

 At the bottom of the form is a blue "Submit" button.

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

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

36.4 Record the ID number on your experiment worksheet [⇒ go to step #1](#)

36.5 Click on Edit Conjugate

Conjugate Details	Delete Conjugate	Edit Conjugate
view	delete	edit

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

Nanodrop post labelling (ug/ml)

495

Volume of labeled antibody (ul)

140

Labeled stock (ug/ml)

200

Dilution factor

2.475

Volume to add (ul)

206.50

Total volume

347

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

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 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