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Antibody lyophilization

Christine Camacho[1], Marc MB Bosse[1], Sean Bendall[1], Mike Angelo[1]
[1]Department of Pathology, Stanford University

1 Works for me dx.doi.org/10.17504/protocols.io.bhmgj43w

Human BioMolecular Atlas Program (HuBMAP) Method Development Community
Tech. support email: Jeff.spraggins@vanderbilt.edu

Christine Camacho

ABSTRACT

This protocol details the lyophilization process of stock antibodies using an overnight lyophilization process.

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MATERIALS TEXT

MATERIALS

D-( )- Trehalose dihydrate **Sigma Aldrich Catalog #T5251-10G** Step 4.1

Ultrapure Water **Thermofisher Catalog #10977023** Step 4.1

Centrifugal Filters 0.1µm **Millipore Sigma Catalog #UFC30VV00**

Millex VV Syringe Filter Unit Filter 0.1µm **Merck Millipore Catalog #SLVV033RS** Step 4.2

FreeZone Lyophilizer Freezer **Contributed by users Catalog #10 400 014**

FreeZone Small Tray Lyophilizer Cabinet **Contributed by users Catalog #10 400 474**

BioCision Cool Box XT System **Corning Catalog #CLS432021**

.5 mL Microcentrifuge Tube with Cap **VWR International Catalog #16466-050**

.5 mL Microcentrifuge Cap **VWR International Catalog #16466-082**

Automatic Pipette E4 XLS (100µL) **Rainin**

CoolRack Modules **Corning Catalog #75779-898** Step 2

Note: CoolRack Modules vary based on quantity of vials you are lyophilizing.

Label templates

1 Templates for the tube labels and dots for the caps can be found here:

UseThisDOT_Letter-48-Template.docx

UseThisLyoTUBELABELTemplate.doc

Preparation 1h

2 If processing a large amount of vials, pre-cool extra aluminum tube racks for at least **01:00:00** at **-80 °C** . 1h

CoolRack Modules **Corning Catalog #75779-898**

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Ice bucket with dry ice and 90 vial rack

3 On the lyophilizer screen menu, press the collector tab and start the collector.

Freezone Small Tray Lyophilizer Cabinet

FreeZone **10-400-474**

Labconco™ FreeZone™ 2.5L -84°C Benchtop Freeze Dryer

FreeZone **10-400-014**

4 Thaw on ice the necessary amount of frozen aliquot 500 mM trehalose solution, or prepare solution fresh

4.1 To prepare fresh trehalose 500 mM solution, in a 50mL conical falcon tube, add **1.89 g** of trehalose and **10 mL** of Ultrapure water.

Falcon 50mL Conical Centrifuge Tubes **Fisher Scientific Catalog #14-432-22**

Ultrapure Water **Thermofisher Catalog #10977023**

D-( )- Trehalose dihydrate **Sigma Aldrich Catalog #T5251-10G**

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4.2 Filter with a syringe filter unit (0.1 µm membrane). Store remaining solution in 1 mL aliquots at **-20 °C** .

Millex VV Syringe Filter Unit Filter 0.1µm **Merck Millipore Catalog #SLVV033RS**

Preparation of 5 µg Antibody Aliquots

5 Select antibodies to lyophilize using data provided on MIBItracker.

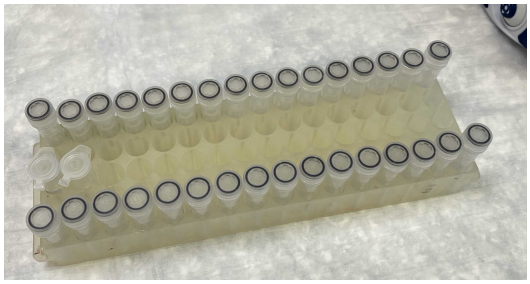
6 Measure the total volume of each antibody to lyophilize using 100-1000µl pipette and record volume. Alternatively, use the total volume (including the antibody stabilizer) recorded in the MIBItracker.

7 Add records on the calculation Excel sheet:
Conjugation ID, Target name, Channel, Concentration, Total volume of labeled antibody to lyophilize

Calculation sheet will give you:
a) number of vials of 5 µg
b) volume of 500 mM trehalose to add to get 100 mM final
c) volume of ultrapure water complete volume of master mix

A	B	C	D	E	F	G	H
ID	Target	Channel	Concentration (µg/mL)	Total volume (µL)	**#vials**	**Trehalose**	**H2O**
1615	NeuN	154	200	791	31.64	632.8	1740.2

8 Using the calculation sheet, prepare and label tubes prior to start. Prepare an ice bucket with dry ice and cooled aluminum rack.



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VWR® SuperClear™ Screw Cap Microcentrifuge Tubes **VWR international Ltd Catalog #16466-050**

9 Aliquot mixture into tubes using automatic pipette. Cap tubes and place into cooled rack to snap freeze. Once you have snap frozen the samples they must be kept frozen.

An automatic pipette is very useful for this protocol, especially if you have a large batch of vials. Set your automatic pipette to aspirate and dispense 100µl.

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Snap Freeze Samples

10 Transfer the samples to **-80 °C** for at least 1 hour. Samples can be held at this step at **-80 °C** for longer if queued for vacuum chamber. Vials may be capped and stored in plastic cryoboxes if desired.

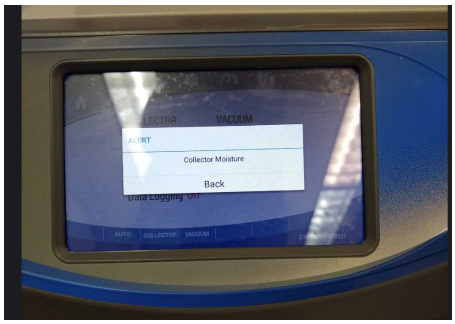
Lyophilization

11 When ready to dry samples, ensure frozen vials are placed into aluminum rack. Place loaded rack with vials into chamber.

12 ⚠

NOTE: If message appears for "Collector Moisture", simply plug hose/bottle located on the left of the machine into the plug located at the left front. Moisture will automatically drain into waste collection bottle attached to the hose.

Unplug collection setup prior to running collector.



13 Ensure release vacuum valve is closed (valve is located on back left side of chamber cabinet). Maximum of two shelves in chamber are allowed. Maximum of 4 x 90 vial racks allowed.

14 ✳

On the lyophilizer screen menu, press the vacuum tab and start the vacuum.
You can record pressure and temperature parameters by pressing the folder icon tab and name a new logging file starting with the date format YY/MM/DD followed by the initial of the user (ex. 180117MB).

Finish process and Storage

15 Allow process to run for 24 hours. End process by selecting "Collector" -> STOP and "VACUUM" -> STOP from the menu.

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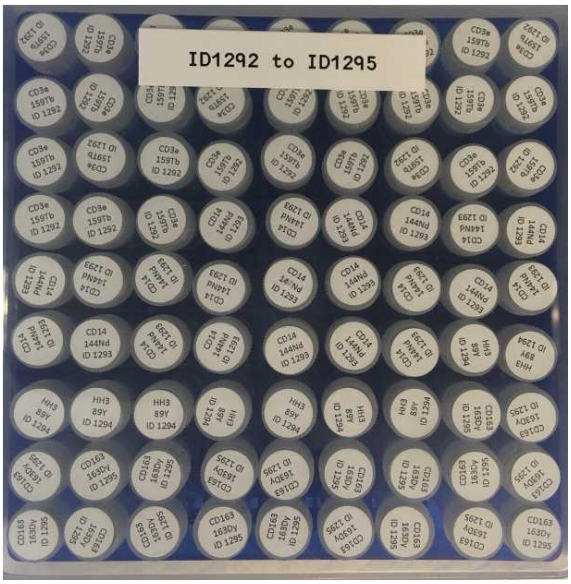
Before collecting samples from vacuum chamber cabinet, obtain new caps for tubes.

16 Open vacuum release valve and open the vacuum chamber. Remove samples. Immediately cap vials with new caps to prevent moisture from getting into the sample.

17 Store at **4 °C**



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