VERSION 2

APR 17, 2024

OPEN ACCESS

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Protocol status: Working
We use this protocol and it's working

Cryomiller SOP V.2

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ABSTRACT

Standard of procedure for cryomiller Retsch at the Soft Materials Laboratory (EPFL)

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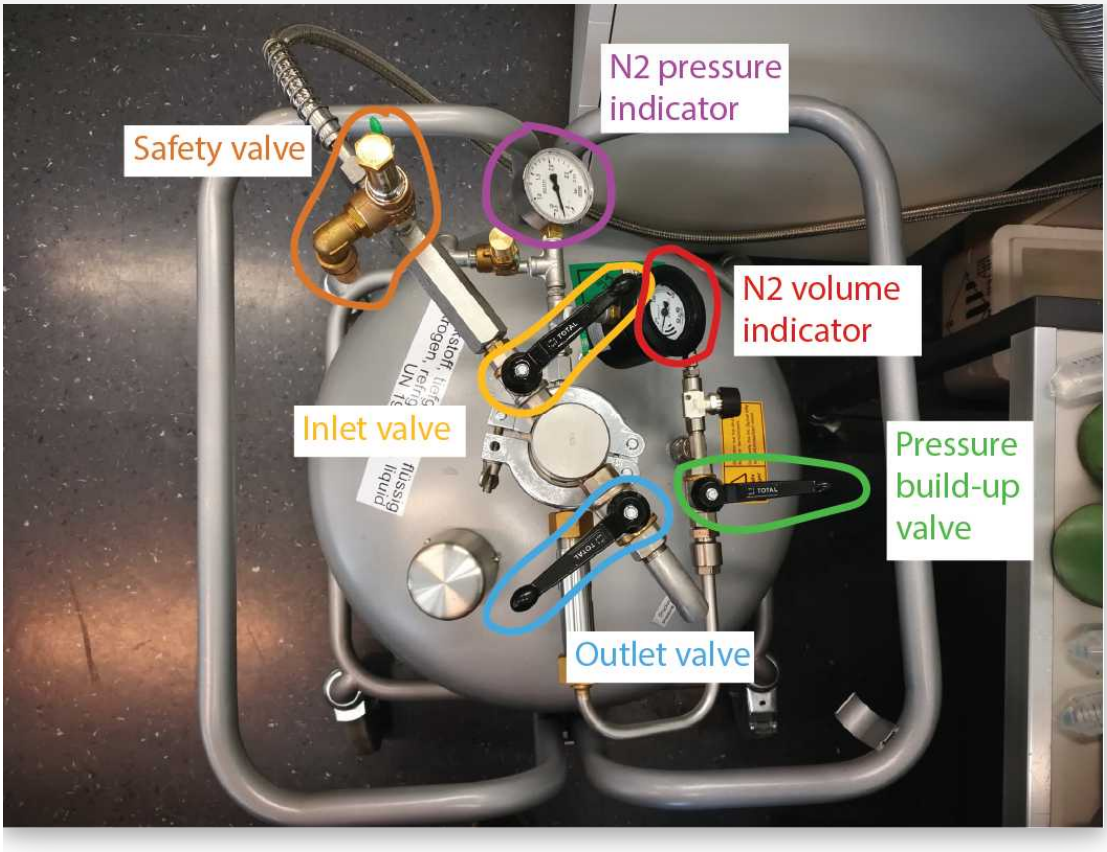
PROTOCOL integer ID: 98320

Know your instrument

1



2

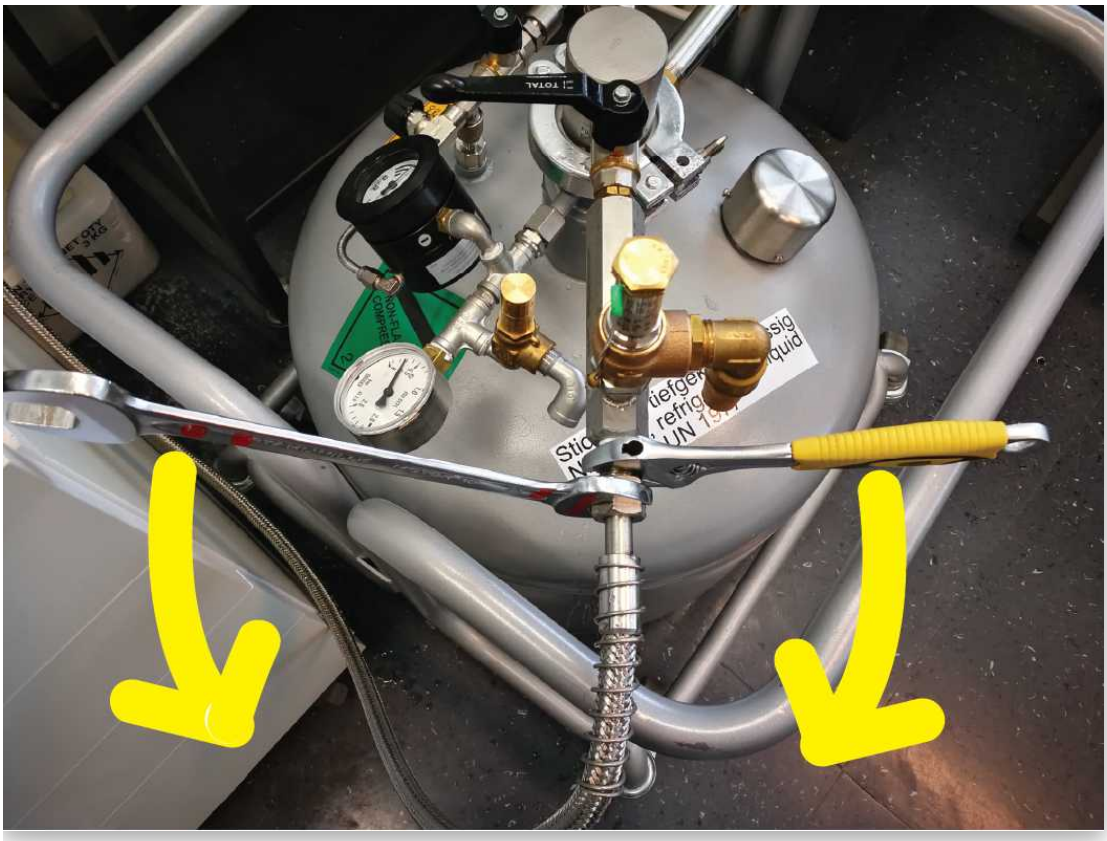
Refill the N2 dewar

30m

3 **Tip:** Refill the dewar at least 2 h before using the cryomiller. Ideally, plan to refill the dewar the day before using the cryomiller.

Refilling in advance allows the pressure inside the dewar to stabilize, avoiding issue during the cryomilling cycles

4 Use two wrenches to unscrew the N2 pipe from the dewar. Firmly push downwards both wrenches at the same time.

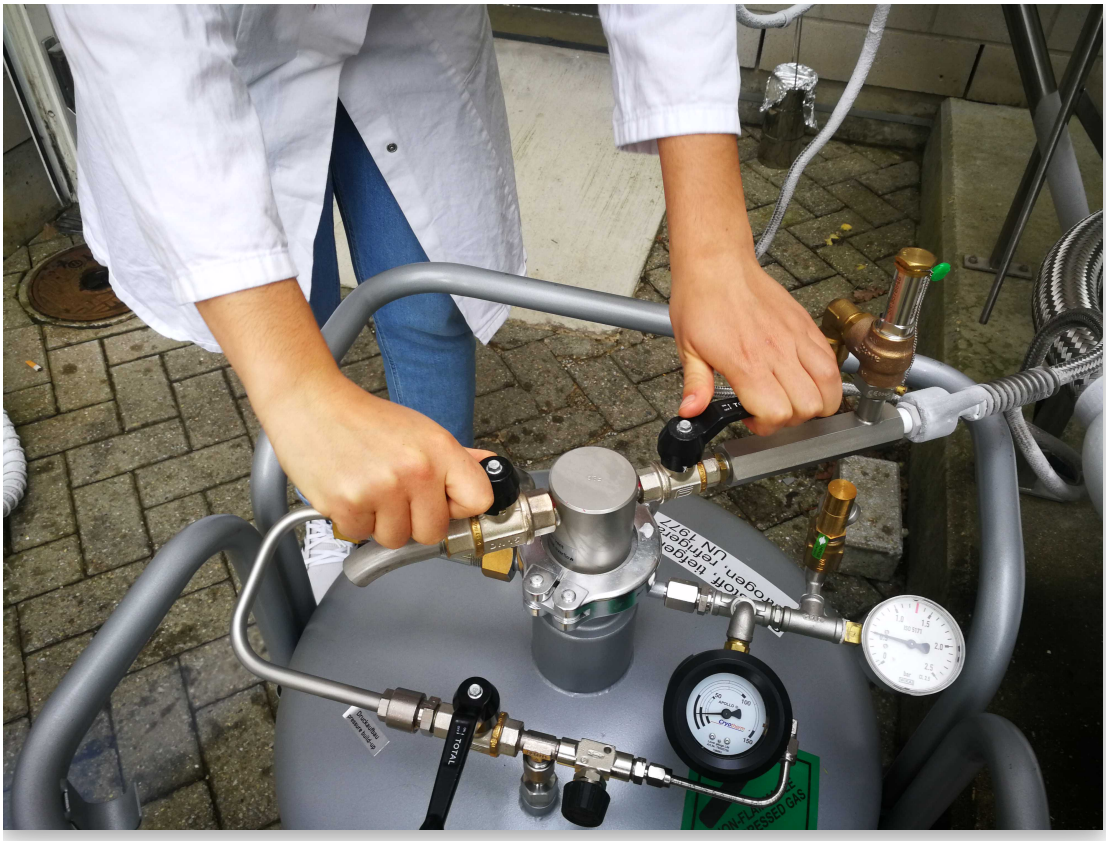


Unscrew the N2 line from the dewar.

5 Place the dewar in the lift and block the wheels. Pull the red strap in front of the door of the lift and send the lift to floor 0.

6 Hand-tight the butterfly screw of the N2 line to the inlet valve of the dewar.

7 Open the inlet and the outlet valve of the dewar.



8 Set the main switch (red) to 1. Then, press the green button. The green button lights up and the line of the N2 reservoir is now open.

9 Turn the tap counterclockwise to start filling up the dewar.

10 Monitor the dewar as it get refilled. The dewar is full when you see some liquid nitrogen coming out of the outlet valve of the dewar (see video).

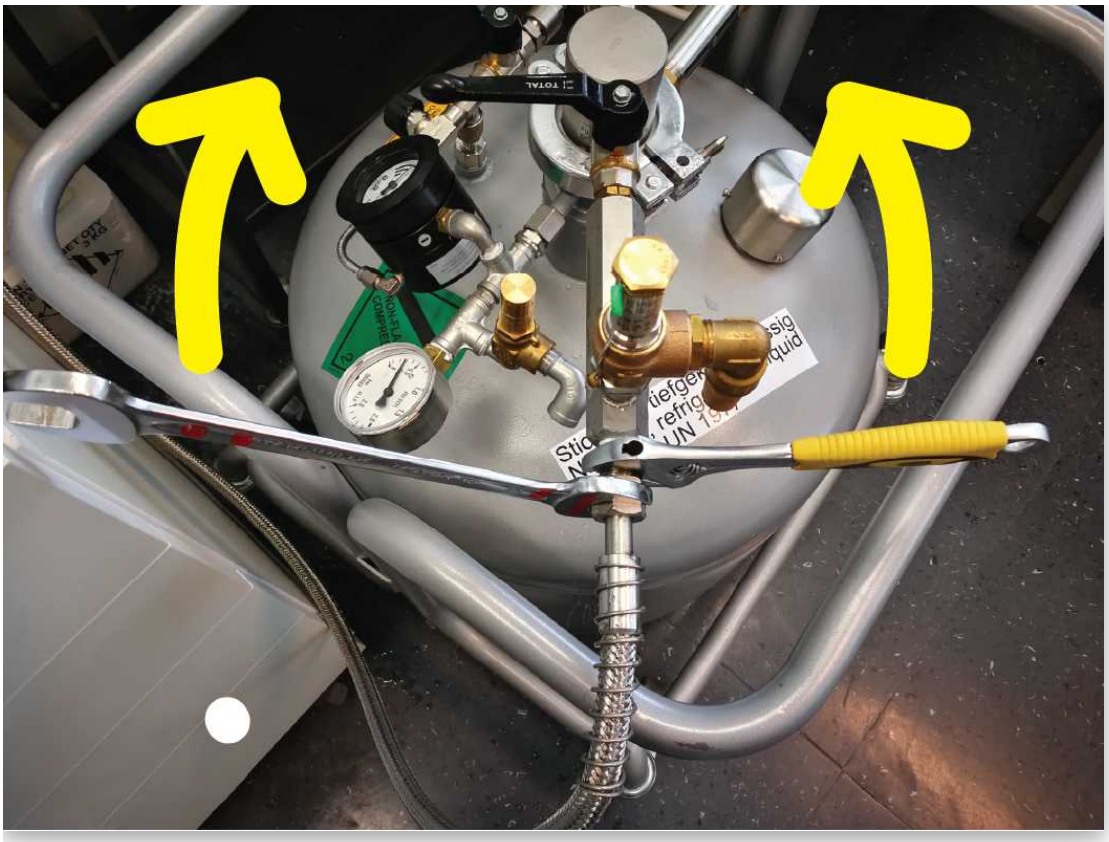
11 When the dewar is full, set the main switch (red) to 0. This step closes the electronic valve between the main reservoir and the filling line.

12 Put on your cryogenic gloves. Close the tap. Then, close the inlet and the outlet valve of the dewar.

13 Unscrew the butterfly screw from the dewar.

Before milling - setup the cryomiller

14 Screw the N2 line to the dewar. Hand-tight the N2 line by using two wrenches. Do not over-tight the bolts.



Screw the N2 line to the dewar.

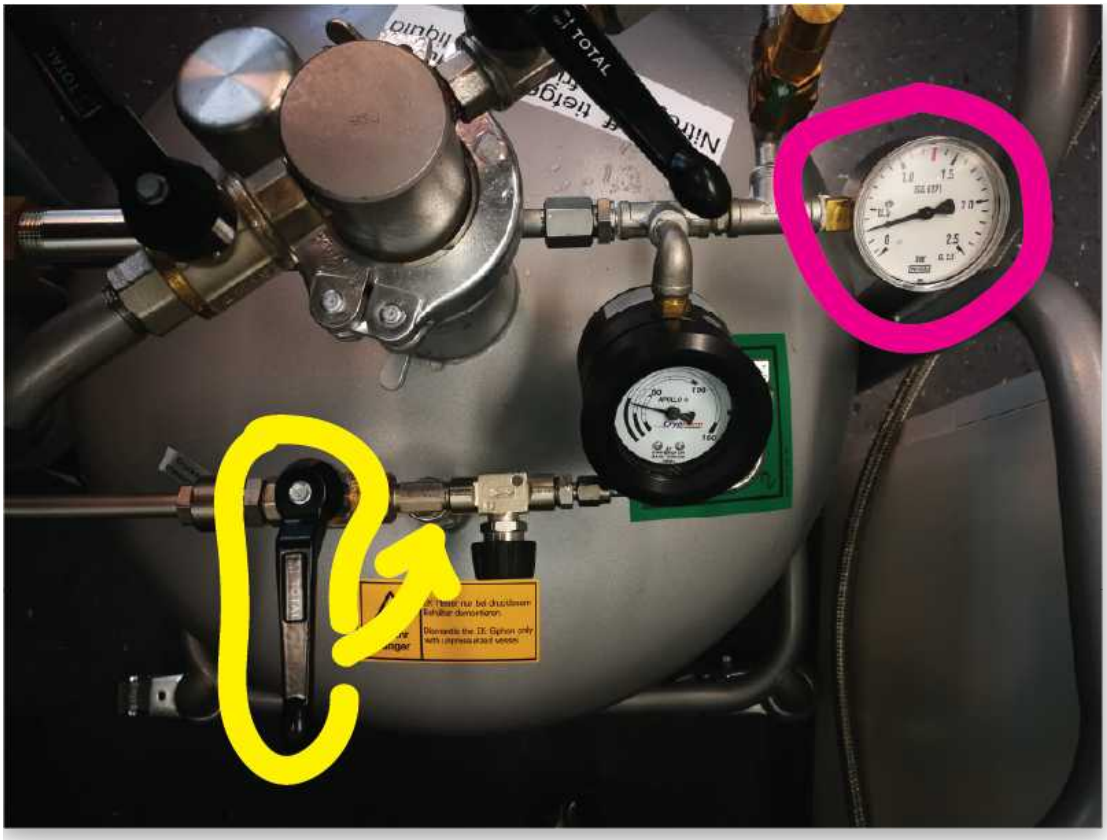
15 Open the inlet valve of the dewar.

16 Build up pressure inside the dewar by slowly opening the pressure build-up valve.

Let the pressure rise to 1 bar, then close the pressure build-up valve. Wait for the pressure to drop below 0.5 bar (~ 10 seconds). Then, slowly open again the pressure build-up valve until you reach 1 bar. Repeat this process for 3 times until the pressure stabilizes around 1 bar.

Note: reaching a stable pressure value may require some hours, due to the pressure feedback system of the dewar. Monitor the internal pressure of the dewar during your first cryomilling cycle.

Tip: refill the dewar at least 2 h before using the cryomiller, to allow pressure to stabilize.



17 Switch on the cryomiller.

Prepare the sample

18 The milling jar volume is 50 mL. The sample to be milled should occupy maximum **about 1/3 of the total jar volume.** For efficient milling, introduce 10 mL to 20 mL of sample in the milling jar.

19 If your material can be prepared in smaller pieces, cut it or break it to facilitate the milling process.
Example: Materials like paper, plastic films, and hydrogels can be cut into small pieces by scissors.

Filling the milling jar

20 **Tip:** 1/3 of the volume filled with the sample, 1/3 of milling balls, 1/3 of free space.

Choose the right milling ball

21 **Tip:** use a milling ball whose diameter is about **3x the size of the biggest particle** composing your sample.

Setup the cryomilling program

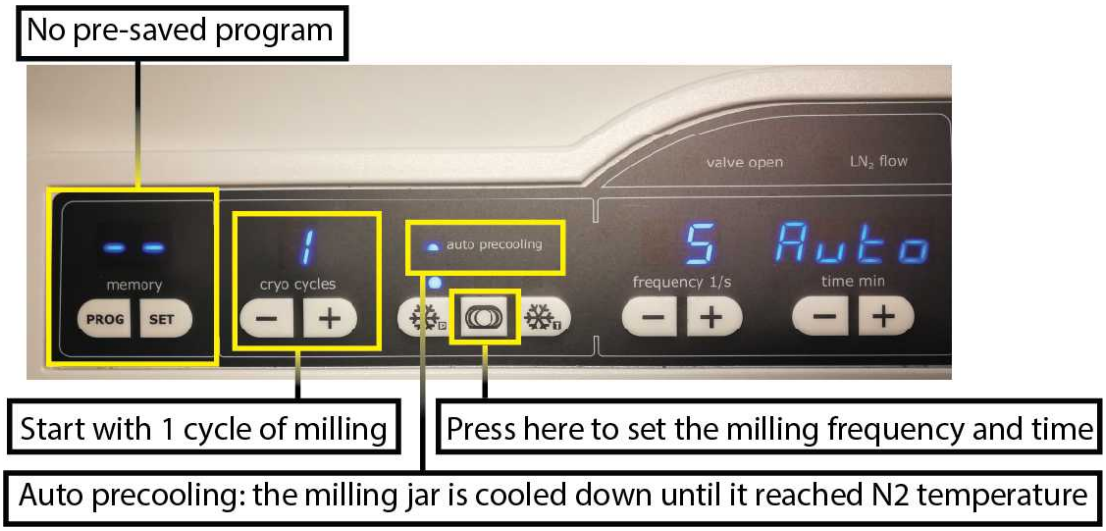
22 Have you already used the cryomiller?

STEP CASE

Quick and dirty - First time user 2 steps

I have never milled my samples before and I have no idea of which parameters to use. Firstly, I want to get an idea of how the milling process works on my samples and then I will optimize my protocol.

23 To run a quick and dirty test, use the **"--" program**: this is a blank memory slot that allows you to try a quick test without saving the milling parameters.


No pre-saved program
valve open
LN_2 flow
memory
PROG
SET
cryo cycles
auto precooling
frequency 1/s
time min
Start with 1 cycle of milling
Press here to set the milling frequency and time
Auto precooling: the milling jar is cooled down until it reached N2 temperature

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