



Cryo-EM Merit Badge Independence Checklist

A Collaboration Among the NIH-Funded Cryo-EM National Centers

Refeyn TwoMP Independence Test Checklist – CryoEM Merit Badges

Trainee: _____

Inspect and clean Objective Lens (Note: at some centers, staff will clean the objective lens)

- ☐ ☐ Clean objective lens, according to center instructions (quick isopropanol wipe or thorough cleaning if needed)
- ☐ ☐ **NEVER** use a Kim wipe to clean the objective.

Turn on Instrument

- ☐ ☐ Power on the instrument electronic controller box.
- ☐ ☐ Confirm power to the instrument by checking for the red positioning light in the objective lens.
- ☐ ☐ Turn on the anti-vibration table (Power and Isolation button, if relevant).
- ☐ ☐ Open the Acquire^{MP} software from the support computer Desktop.
- ☐ ☐ Confirm connection to instrument by high-pitched sound or green laser on MP (open the lid very slightly).
- ☐ ☐ Wait ~1 hour before use.
- ☐ ☐ Demonstrate how to open the user manual and help menu.

Prepare Slides

- ☐ ☐ Use new pre-cleaned cover slides or slides freshly cleaned with isopropanol and water and allowed to dry.
- ☐ ☐ Use new or freshly cleaned gaskets.
- ☐ ☐ Assemble slides using a clean alignment tool, removing bubbles under the gasket by pressing gently with forceps or pipette tip between the wells.
- ☐ ☐ Keep slides clean by holding only the edges.
- ☐ ☐ Keep slides in a closed box until use.

Prepare Buffer and Sample

- ☐ ☐ Prepare sufficient buffer and sample at a sensible concentration.
~ 20 μ L buffer and ~2 μ L sample per measurement, with a sample concentration ~100 nM before dilution into the sample well.
- ☐ ☐ Allow sample and buffer to equilibrate to room temperature before measurement.
 - ☐ ☐ If the sample is temperature sensitive, keep on ice until ready to use.

Add Immersion Oil to the Objective Lens (note some centers require staff to handle immersion oil)

- ☐ ☐ Place a single drop of immersion oil, with no visible bubbles, on top of the lens.
- ☐ ☐ DO NOT touch the objective lens with the plastic tip of the applicator.
- ☐ ☐ DO NOT allow any oil to run down the side of the objective lens.

Seat the Slide + Gasket

- ☐ ☐ Place the slide, gasket-side-up, in the holder above the objective lens.
- ☐ ☐ Verify there are no air bubbles in the oil. If there are, reapply.
- ☐ ☐ Secure slide in place with provided magnets on opposite corners.

Align the First Well

- ☐ ☐ Use the Acquire^{MP} stage control window to center the upper left well, and click “Set Position”.



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Recording a measurement

- ☐ ☐ Add room temperature sample buffer to the aligned well (between 10-20 μ L).
- ☐ ☐ Perform focus routine using the Acquire^{MP} software, and confirm good focus and clean glass.
- ☐ ☐ If not good, move the new area or new well until you have a good focus on clean glass.
- ☐ ☐ If desired: Record a measurement in Acquire^{MP} from buffer to check for suitably low background counts.
- ☐ ☐ Add room temperature sample to the buffer drop, and mix thoroughly without touching slide.
- ☐ ☐ Close lid and visually confirm event density is appropriate using the ratiometric view.
- ☐ ☐ Click "Record" in Acquire^{MP}.
- ☐ ☐ Save the recording in the appropriate location per center instructions.

If Generating A Calibration Curve

- ☐ ☐ Prepare standard according to product manual, if possible, in your sample buffer.
- ☐ ☐ Collect a recording using the standard sample as described above. Save the data in the appropriate location.
- ☐ ☐ Open the Discover^{MP} software and open the calibration measurement.
- ☐ ☐ Select 'Create' under the calibration menu and choose your measurement.
- ☐ ☐ Assign the proper masses to your resulting peaks according to the manufacturer's values.
- ☐ ☐ Assess calibration fit: R^2 value should be 0.99 - 1.00.
- ☐ ☐ If using results to determine MW, make a new calibration curve every ~3 h.

Assessing the data

- ☐ ☐ Open the recorded data in the Discover^{MP} software.
- ☐ ☐ Double click on the calibration curve (or one opened from a previous run) to convert units to mass.
- ☐ ☐ Demonstrate ability to navigate Discover^{MP}.

Taking additional measurements

- ☐ ☐ Repeat the above steps as needed to complete desired measurements.
- ☐ ☐ Oil is cleaned and reapplied to objective lens every ~45 minutes or when changing to a fresh slide.

Cleaning up

- ☐ ☐ Unlock the focus in Acquire^{MP} and center the stage.
- ☐ ☐ Remove the magnets, and used slide from the stage.
- ☐ ☐ Place slides and gaskets in the appropriate place for disposal/cleaning.
- ☐ ☐ Clean the objective lens as per center instructions, until no residual oil is on the objective.
 - ☐ ☐ Note: at some centers staff will clean the objective lens.

Turn Off the Instrument (If you are the last user of the day. If not, leave on.)

- ☐ ☐ Close Acquire^{MP} software.
- ☐ ☐ Turn off anti-vibration table (isolation and power button if relevant).
- ☐ ☐ Turn off instrument (Electronic Controller power switch).

Evaluator Notes:

Supervising Staff: _____

Date: _____

Supervising Staff: _____

Date: _____