



Refeyn TwoMP Independence Test Evaluator Checklist – CryoEM Merit Badges

Supervising Staff: _____ Date: _____

Trainee: _____

Prepare Objective Lens

Note: at some centers it will be staff who clean and apply the immersion oil to the objective lens

- If cleaning lens, cut a small square piece of lens paper
- Apply ~20 uL of isopropanol onto the lens paper
- Wipe the objective lens in a circular motion starting from the center
- Repeat steps 1a-c until the isopropanol evaporates from the objective lens
 - Repeat cleaning every 30-45 min during instrument use
 - NEVER** use a Kim wipe to clean the objective

Turn on TwoMP Instrument

- Instrument needs to be turned on ~1 hour before use
- Flip button on the Electronic Controller under the monitor
- Press Power button on the anti-vibration table
- Press Isolation button on the anti-vibration table
- Sample well cassettes (gaskets) (Refeyn MP-CON-21022)
- Open the Acquire^{MP} program from the Desktop

Evaluator Notes:

Prepare Buffer and Sample

- If needed, prepare any dilutions or spin down sample
 - The final volume for sample measurements will be 16-20 ul
 - We typically add 2uL sample into 14-18 ul buffer
- Final sample concentration must be within 5-20 nM
- Your sample and buffer should be at room temperature
 - We typically add 2uL sample into 14-18 ul buffer
 - If temperature sensitive, keep sample on ice until ready to use

Evaluator Notes:

Prepare the Slide

- Acquire new or pre-cleaned cover slides and gaskets



- Inspect slide for dust
 - If there is dust on the slide, do not use
 - Refer to Refeyn’s recommended slide cleaning protocol if needed
- Inspect alignment tool et alignment tool - if dusty, clean with isopropanol and dry
- Peel off a silicon gasket from backing with forceps
- Fit gasket into center of alignment tool
- Place the cover slide into the alignment tool, hold slide by the edges only
 - If you see bubbles, tap slide gently with forceps or pipette tip between the wells to remove

Evaluator Notes:

Add Immersion Oil to the Objective Lens

- Open the Zeiss immersion oil bottle
- Let the drop form from the tip of the applicator
- Let single drop fall into the objective lens
 - DO NOT touch the objective lens with the plastic tip of the applicator
 - This could damage the objective lens (very expensive)

Seat the Slide + Gasket

- Place the slide, gasket-side-up, in the holder above the objective lens
- Secure slide in place with provided magnets on opposite corners

Evaluator Notes:

Align the First Well

- Go to Acquire^{MP} stage control window
- Change stage speed to 50X
- Use arrows in Acquire^{MP} to center the light in the upper left well
- Select upper left well and click “Set Position”

Evaluator Notes:

Make Calibration Recording

- Prepare a 1:50 dilution of standard, either NativeMarkTM Unstained Protein Standard or MassFERENCE P1 standard, in your sample buffer
- Add 18ul of your sample buffer to the first well
- Perform focus routine per software instructions



- Carefully add 2 ul of the 1:50 dilution of your standard to the buffer in the first well, then gently mix with larger pipette 5-6 times
- Start measurement in Acquire^{MP} by hitting “Record”
- Save your recording in the appropriate location per center instructions

Evaluator Notes:

Generate Calibration Curve

- Open the Discover^{MP} software
- Open your calibration measurement by hitting the “+” button in the upper left
- Select ‘Create’ under the calibration menu and choose your results
- Assign the masses to your resulting peaks according to the manufacturer’s values
 - Note: Refeyn suggests that the R² value should be 0.99 - 1.00

Evaluator Notes:

Make Sample Measurements

- In Acquire^{MP}, move stage to next clean well
- Add your appropriate volume of buffer at room temperature
- Click “Droplet Dilution” to focus
- Pipet appropriate volume of test sample into buffer droplet
- Pipet 5-6 times with larger pipette to mix without touching slide
- Verify that ratiometric view looks appropriate
- Click “Record”
- Save data in your project folder
- Repeat the above steps as needed to complete all desired measurements
 - Oil should be cleaned/reapplied to objective every ~45 minutes

Evaluator Notes:

When Finished with a Session

- Unlock the focus in Acquire^{MP}
- Remove the magnets, slide and gasket from the stage
- Place slides and gaskets in appropriate place for disposal

Clean the Objective Lens

Note: at some centers it will be staff who clean and apply the immersion oil to the objective lens; ONLY use the



provided lens cleaning paper

- Cut lens paper or fold into a thin rectangle
- Use a first piece to remove bulk excess oil from objective lens
 - Do not get oil on the stage; if so, clean with isopropanol
 - Add isopropanol (~20 ul) to second piece of lens paper and wipe the objective lens in a circular motion outward from the center of the lens
- Repeat with fresh lens paper until isopropanol is evaporating as soon as you start wiping

Evaluator Notes:

Turn Off the Instrument

- If you are the last user of the day, turn the instrument off in the opposite order in which things were turned on
 - Turn off Isolation button on anti-vibration table
 - Turn off Power button on anti-vibration
 - Turn off Electronic Controller power switch

Evaluator Notes:
