1. General Guidelines

Refer to these guide lines when running or working at the liquid handlers. See the Tecan policy document for an extended version covering most robot room aspects.

1. Turn on the liquid handler.
    a) Make sure that the waste tube has a free way to the waste container and that the waste container is not full.
    b) Make sure the system liquid is not empty.

2. Start the computer (windows password: "freedomevo").

3. Start EVOware
    a) Log in as "IFuser" or "IFapplication" (password: "greenbluered").
    b) Choose "Run an existing script" and chose the desired script from the selection window list. All relevant scripts have the prefix "IF_".
    c) Toggle the runtime controller extended view to display the work table and to double check that the intended script was chosen (top comment row).

    NB: If the liquid handler is used for the first time in a session, choose "Run maintenance" instead and run the script "morgontvatt" before running production scripts.

4. Setup the physical worktable to look exactly like the one in EVOware and/or LIMS.
    a) Move carriers to match the worktable. Remove all other carriers.
    b) Situate your lab ware in the correct positions.
    c) Mount the correct LiHa-tips in the correct positions and/or place the correct amount of nested tip racks at the correct MCA tip positions.

5. Run the script
    a) Press the "RUN" button.
    b) Read prompts, if any, and make adaptations if necessary.
    c) Scripts are finished when the (green) FreedomEVO status light shuts off.

    NB: The extended view displays the worktable and allows script execution monitoring. If the liquid handler is to run the first script of a session, it will carry out a ca. 30 s long initialization.

6. After a run
    a) Remove all lab ware. Exchange the tip waste container and empty the liquid waste if necessary.
    b) Exit EVOware. (important to avoid (accidental) script changes by unauthorized personel).
    c) Delete work lists (if any) from the desktop.
    d) Turn off the liquid handler.
    e) Make a note with date, name and a message in the log book if something out of the ordinary occurred during the run.

2. Antibody Transfer

1.1 Preparations

1. Mark the four IF_P plates with lowest numbers and press "Move to IF_384" in the "IF Storage" lims-section. Print and attach the IF_384 plate labels.
2. Retrieve the IF_P plates from the freezer and allow them to equilibrate in room temperature for at least 15 minutes. Briefly spin down the source plates.
3. Confirm that the work order specifies alternating and increasing *uneven* IF_P plates for transfer to IF_384 positions 1 - 16 and, alternating and increasing *even* IF_P numbers are designated for transfer to IF_384 positions 17 - 32.

1.2 At the liquid handler

1. Open the script "IF_ab_transfer".
2. Position plates according to the EVOware workspace and start the script.

NB: It is very important that the LiHa:s are sufficiently primed (morgontvatt run) and that all gold cones are tightened before running transfer scripts to avoid loss of the newly delivered antibody.

3. Antibody Dilution

3.1 Preparations

1. Choose the amount of portions to be diluted and print out the dilution work order and labels from LIMS.
2. Retrieve all needed 384-well plates from the freezer and allow them to equilibrate at room temperature for at least 15 minutes.
3. Prepare stock solution as specified by the work order. Spin down the antibody marker tubes before opening.
4. Briefly spin down the 384 well plates in the plate centrifuge.

NB: Be sure to remove bubbles from the buffer tubes as they might otherwise cause incorrect automatic pipetting. Always check the first 200 µl tip buffer aspiration.

3.2 At the liquid handler

1. Make sure old work lists from earlier runs are deleted from the desktop.
2. Download the work list from LIMS and save it to the desktop.
3. Rename it to *worklist_dilution.gwl* and save a copy of the file under Desktop/IF.
4. Run the "IF_Dilution" script.

3.3 Details

tip consumption
- 50 µl LiHa: 95 tips
- 200 µl LiHa : 12 tips

running time (95 antibodies)
~ 40 min

4. Storage to Research

4.1 Preparations

1. Search for an entered list of antibodies and choose “Dilute” or “Aliquot”. Edit the list to set up the desired template scheme.
2. Place the order (make adaptations if prompted to do so), and print the target plate labels.

NB: The minimum aliquotation volume is 10 µl.

4.2 At the liquid handler

See section 3.2.

NB: Both aliquotation and dilution storage to research work orders are executed using the “IF_Dilution” script.

5 Immunostaining

3.1 Preparations

NB: For details on the buffer preparation, see section 5 in the production SOP.

1 plate and 1 template *Reagents needed for Day 1*	1 plate and 1 template *Reagents needed for Day 2*
- 1 glass bottom plate with cells - 2 tip racks - PBS: 150 µl in square deep well plate - TRITON: 200 µl in square deep well plate - WASTE: square deep well plate - PFA: 50 µl in skirted micro plate - PRIM. AB: 40 µl in skirted micro plate	- 1 glass bottom plate with cells - 1 tip racks - PBS: 400 µl in square deep well plate - GLY/PBS: 400 µl in square deep well plate - WASTE: square deep well plate - DAPI: 50 µl in skirted micro plate - SEC. AB 40 µl skirted micro plate

2 plates and 2 templates *Reagents needed for Day 1*	2 plates and 2 templates *Reagents needed for Day 2*
- 2 glass bottom plates with cells - 3 tip racks - PBS: 250 µl in square deep well plate - TRITON: 350 µl in square deep well plate - WASTE: square deep well plate - PFA: 100 µl in skirted micro plate - 2 x PRIM. AB: 40 µl in skirted micro plates	- 2 glass bottom plates with cells - 6 tip racks - 2 x PBS: 400 µl in square deep well plate - 2 x GLY/PBS: 400 µl in square deep well plate - WASTE: square deep well plate - DAPI: 100µl in skirted micro plate - SEC. AB 80 µl in skirted micro plate

3 plates and 3 templates *Reagents needed for Day 1*	3 plates and 3 templates *Reagents needed for Day 2*
- 3 glass bottom plates with cells - 5 tip racks - PBS: 350 µl in square deep well plate - TRITON: 450 µl in square deep well plate - WASTE: square deep well plate - PFA: 150 µl in skirted micro plate - 3 x PRIM. AB: 40 µl in skirted micro plates	- 3 glass bottom plates with cells - 10 tip racks - 3 x PBS: 400 µl in square deep well plate - 3 x GLY/PBS: 400 µl in square deep well plate - WASTE: square deep well plate - DAPI: 150 µl in skirted micro plate - SEC. AB 120 µl in skirted micro plate

2 plates and 1 template *Reagents needed for Day 1*	2 plates and 1 template *Reagents needed for Day 2*
- 2 glass bottom plates with cells - 2 tip racks - PBS: 250 µl in square deep well plate - TRITON: 350 µl in square deep well plate - WASTE: square deep well plate - PFA: 100 µl in skirted micro plate - PRIM. AB: 80 µl in skirted micro plates	- 2 glass bottom plates with cells - 3 tip racks - PBS: 800 µl in square deep well plate - GLY/PBS: 800 µl in square deep well plate - WASTE: square deep well plate - DAPI: 100 µl in skirted micro plate - SEC. AB 80 µl in skirted micro plate

4 plates and 4 templates *Reagents needed for Day 1*	4 plates and 4 templates *Reagents needed for Day 2*
- 4 glass bottom plates with cells - 6 tip racks - PBS: 450 µl in square deep well plate - TRITON: 600 µl in square deep well plate - WASTE: square deep well plate - PFA: 180 µl in skirted micro plate - 4 x PRIM. AB: 40 µl in skirted micro plates	- 4 glass bottom plates with cells - 14 tip racks - 4 x PBS: 400 µl in square deep well plate - 4 x GLY/PBS: 400 µl in square deep well plate - WASTE: square deep well plate - DAPI: 200 µl in skirted micro plate - SEC. AB 160 µl in skirted micro plate

5 plates and 5 templates *Reagents needed for Day 1*	5 plates and 5 templates *Reagents needed for Day 2*
- 5 glass bottom plates with cells - 7 tip racks - PBS: 600 µl in square deep well plate - TRITON: 650 µl in square deep well plate - WASTE: square deep well plate - PFA: 200 µl in skirted micro plate - 5 x PRIM. AB: 40 µl in skirted micro plates	- 5 glass bottom plates with cells - 16 tip racks - extra micro plate carrier - 5 x PBS: 400 µl in square deep well plate - 5 x GLY/PBS: 400 µl in square deep well plate - WASTE: square deep well plate - DAPI: 150 µl + 100 µl in skirted micro plates - SEC. AB 120 µl + 80 µl in skirted micro plates

3.2 At the liquid handler

1. Open the script "IF_DayX_Whatman_X_X".
2. Position labware according to the EVOware workspace and start the script.

NB: Use the IF_preDay2_X scripts prior to running Day 2 scripts for multiple plate to fill all PBS and glycerol deep well plates automatically.

3.3 Running Times

Day 1	*Running Time*	*Day 2*	*Running time*
1 plate:	35 min	1 plate:	3.5 h
2 plates:	< 50 min	2 plates:	4 h
3 plates:	1 h+	3 plates:	5 h
4 plates:	1 h+	4 plates:	6 h+
5 plates:	1 h+	5 plates:	7 h+

4 Maintenance and Problem Solving

4.1 Definitions

- carrier: grey metal holders placed on work table
- lab ware: plastic liquid containers placed on carriers
- tips: black conductive tips for LiHa and transparent nested tips for the MCA

volume	*product number*	*box size*
10 µl	10 612 516	2304 (2*96*12)
50 µl	30 032 115	2304 (2*96*12)
200 µl	30 000 627	2304 (2*96*12)
50 µl (nested)	30 038 609	3840 (8*96*5) 30 038 609
200 µl (nested)	30 038 619	3840 (8*96*5)

4.2 Weekly Maintenance

1. Wipe the gold cone with ethanol using a dust-free tissue.
2. Tighten syringes and gold cones.

4.3 Problem Solving

1. Pause/Stop script
   To pause a script, press the pause button on the liquid handler or in the software. The liquid handler finishes the current command before pausing.

2. Running parts of a script
   To run parts of a script, put in the start script line number in collapsed view runtime controller window before pressing ”RUN”.

3. Dropping tips
   Choose edit existing script, pick any script (works when logged in as IFuser) and run the “Drop Tips” Direct Command for LiHa:s or MCA.