Lunaphore COMET Emulator Software

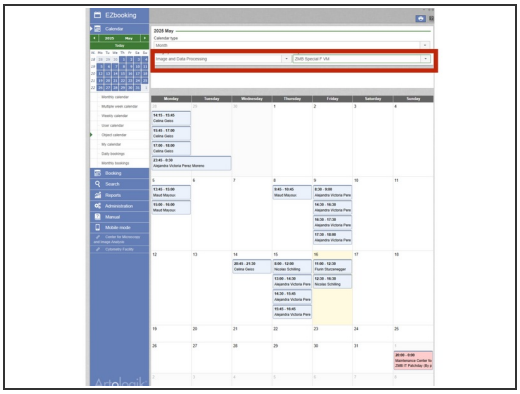
Written By: z mbstaff

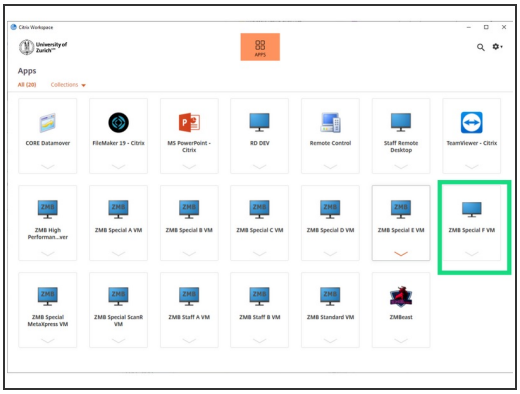


INTRODUCTION

The emulator helps save time during the actual COMET session by allowing you to pre-design protocols and prepare XML files. Based on the Preparation PDF, all antibodies must be pre-diluted and brought in 2 mL Eppendorf tubes only, which are required for use with the COMET.

Step 1 — Start Special F VM via CITRIX

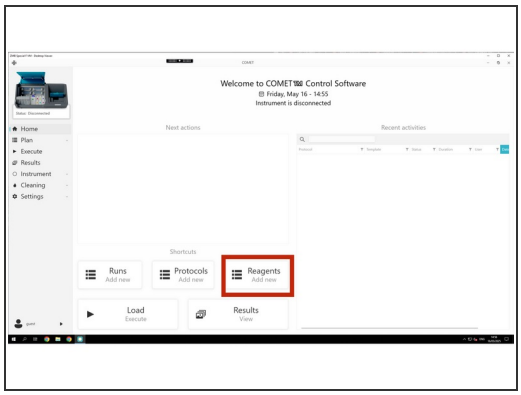


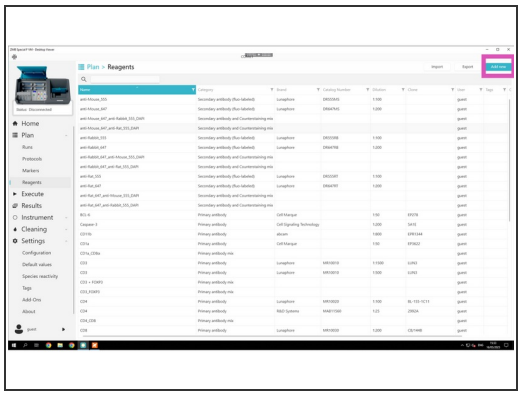


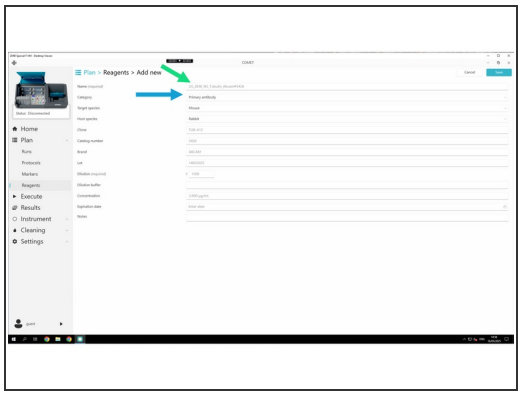


- Book **Special F VM** via https://booking.core.uzh.ch/
- Access CITRIX via app or via broweser https://vdi.core.uzh.ch/ and launch **Special F VM**.
- Start **COMET** control software by double-clicking on the icon.

Step 2 — Create Primary antibody





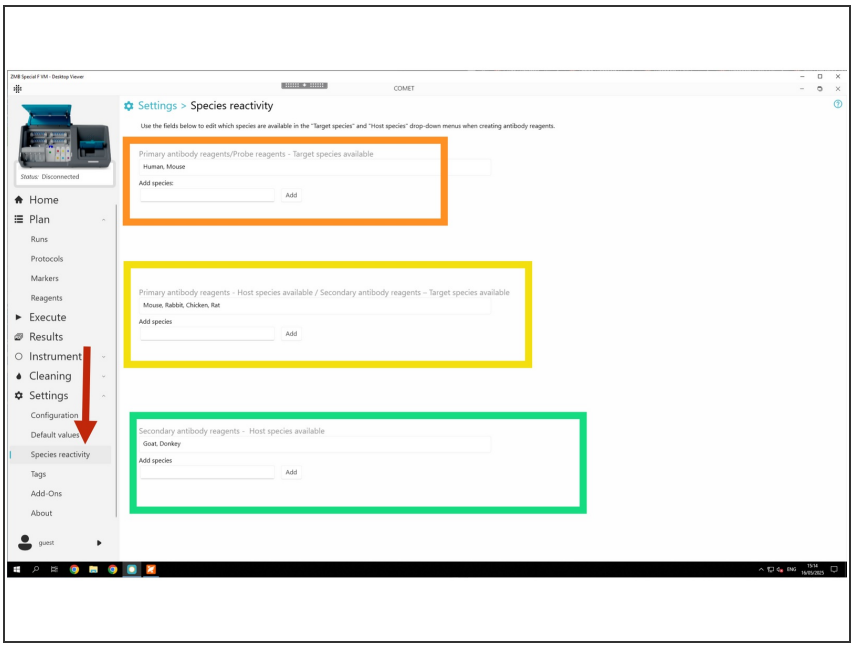


- Click **Reagents**.
- Click **Add new**
- Start creating new primary antibody - here an example
- Follow the required naming convention: **PI initials_Lab_User initials_Antibody_Company_Catalog#_Extra info**

ⓘ *If a host or target species is missing for your antibody, follow these steps to add it (follow step 3)*

ⓘ *For each new reagent, fill in the required fields: Name, Category, and, if applicable: Channel, Dilution, Target genes, and Tails.*

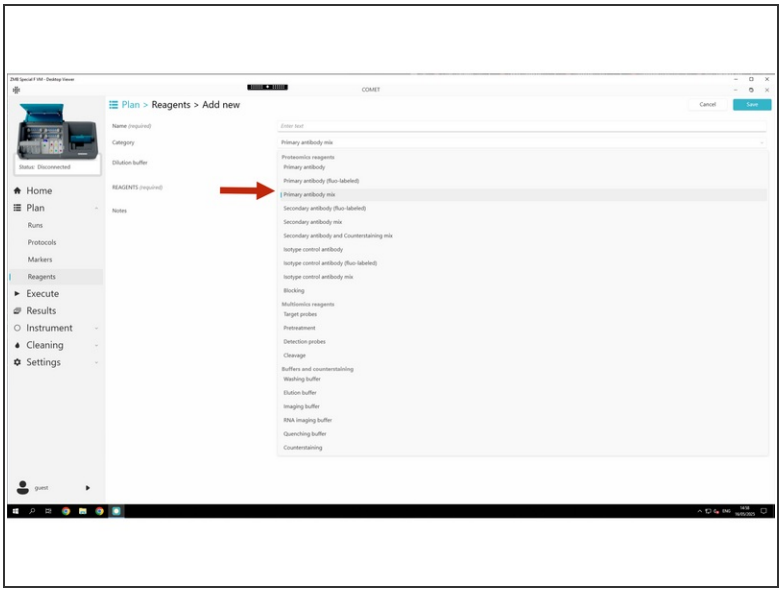
Step 3 — (optional) Adjust Species Reactivity Settings

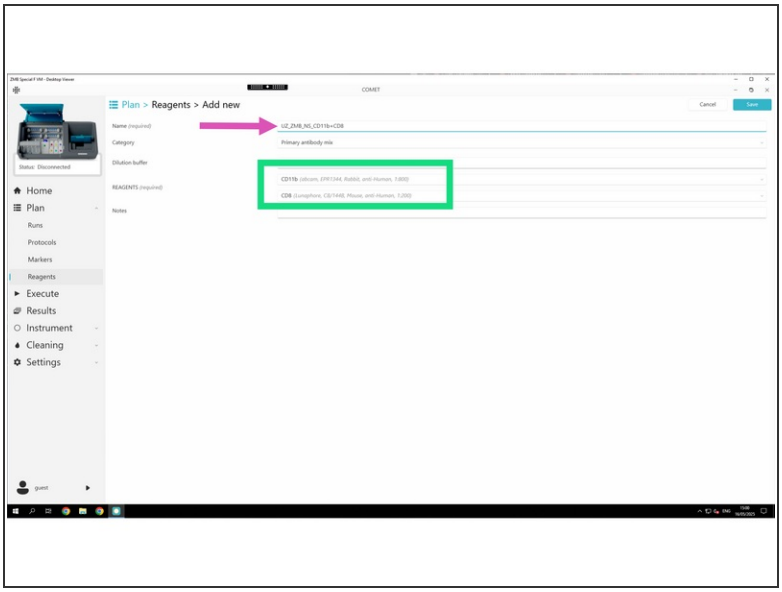


ⓘ *If a host or target species is missing for your antibody, follow these steps to add it.*

- **Settings** → Species reactivity
- Configure Target Species for Primary Antibodies / Probes
- Configure Host Species for Primary and Secondary Antibodies
- Configure Host Species for Secondary Antibodies

Step 4 — Create Primary antibody mixes

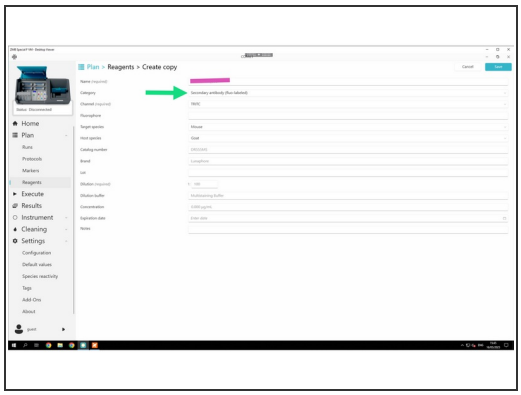
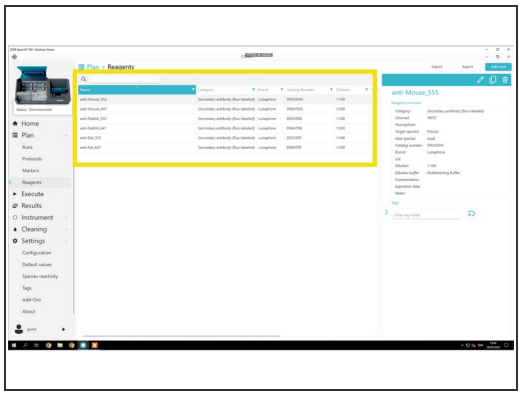
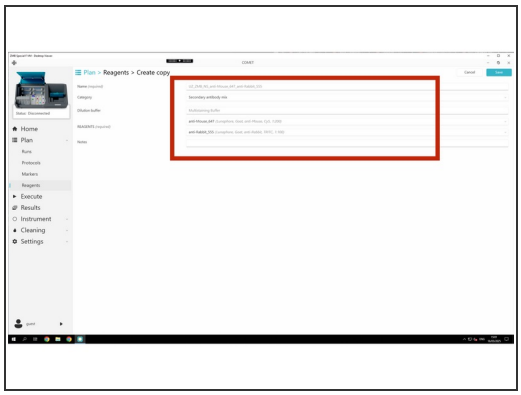




- Once you have all primary antibodies created. You can now create the mix of two primary antibodies which will be than used in the protocol.
- Follow the required naming convention: **PI initials_Lab_User initials_Antibody_Pair_cycle#**
- The software will only allow to combine two different species for obvious reasons.

This document was generated on 2026-04-17 09:18:46 PM (MST).

Step 5 — Create individual Secondary antibody and mixes

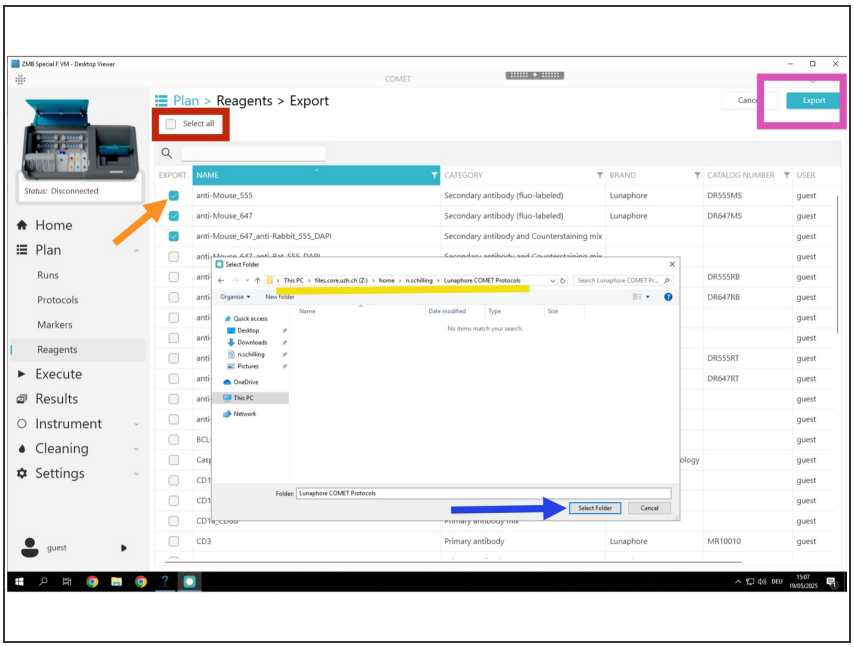
- Create your individual secondary antibodies.
  - Follow the required naming convention: **PI initials_Lab_User initials_Antibody_Company_Catalog#_Extra info**

ⓘ *For each new reagent, fill in the required fields: Name, Category, and, if applicable: Channel, Dilution, Target genes, and Tails.*

- Adapt dilution for secondary antibodies accordingly.
- After creating your individual secondary Antibodies - create mixes of secondary antibodies.
  - Follow the required naming convention: **PI initials_Lab_User initials_Secondary_Pair_i.e. Pair_dk_anti_ms_AF555_dk_anti_rb_AF647**

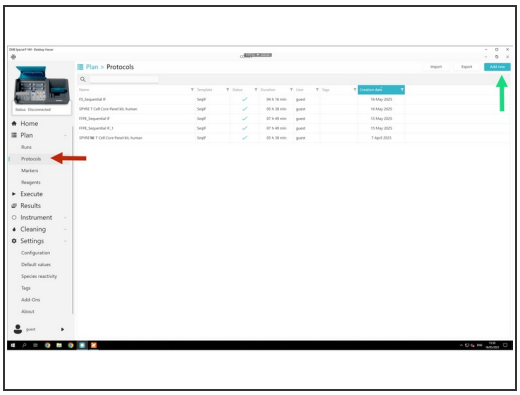
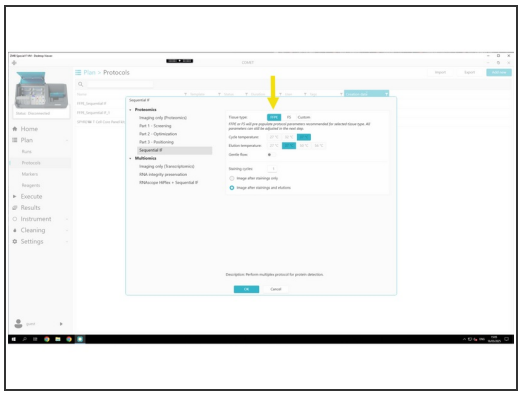
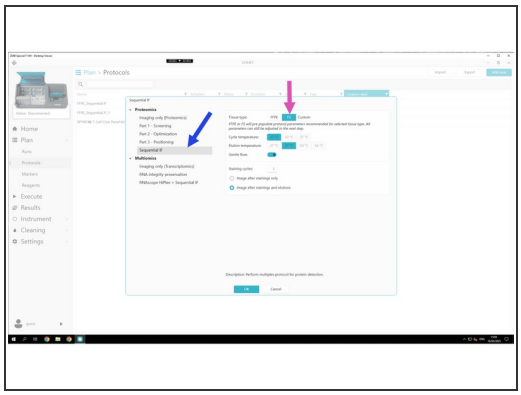
⚠ Lunaphore secondary antibodies must be used unless discussed beforehand. Stick to Alexa Fluor™ Plus 488, 555, 647, or 750.

Step 6 — Exporting Reagents



- Click **Export**
  - Use the checkboxes to select individual reagents.
  - Or use Select All if you want to export the entire reagent library.
- Creat a new folder "Lunaphore COMET Protocols" in Z:\home\u.ser\Lunaphore COMET Protocols Export the selected reagents to Z:\home\u.ser\Lunaphore COMET Reagents
- Click **Select Folder** in order to save it as an .xml file

Step 7 — Create Protocol

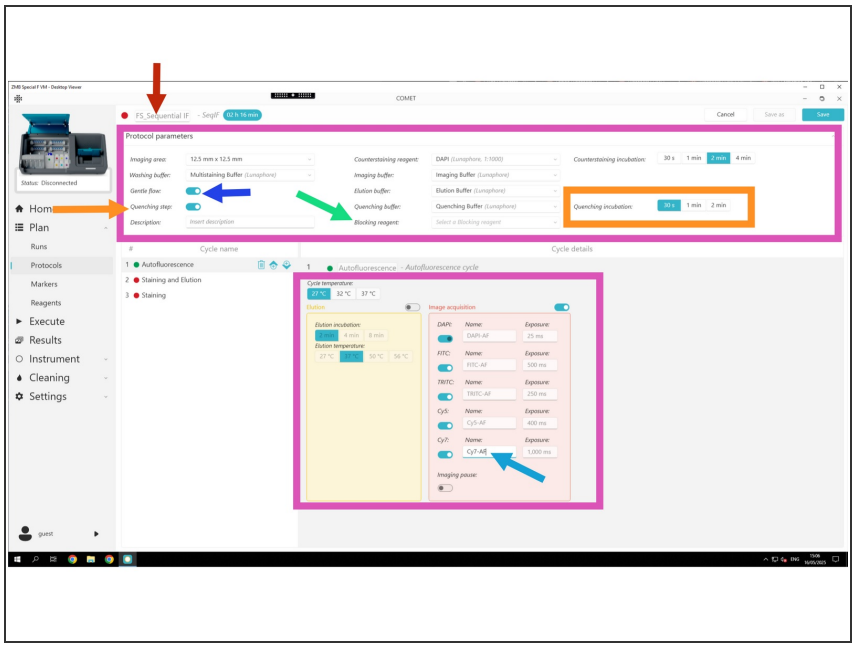




- Click **Protocols**.
- Click **Add New**.
- Select **Sequential IF** template.

Select your tissue type (When selecting FFPE or FS, default protocol parameters are automatically optimized, so you typically don't need to change anything.):

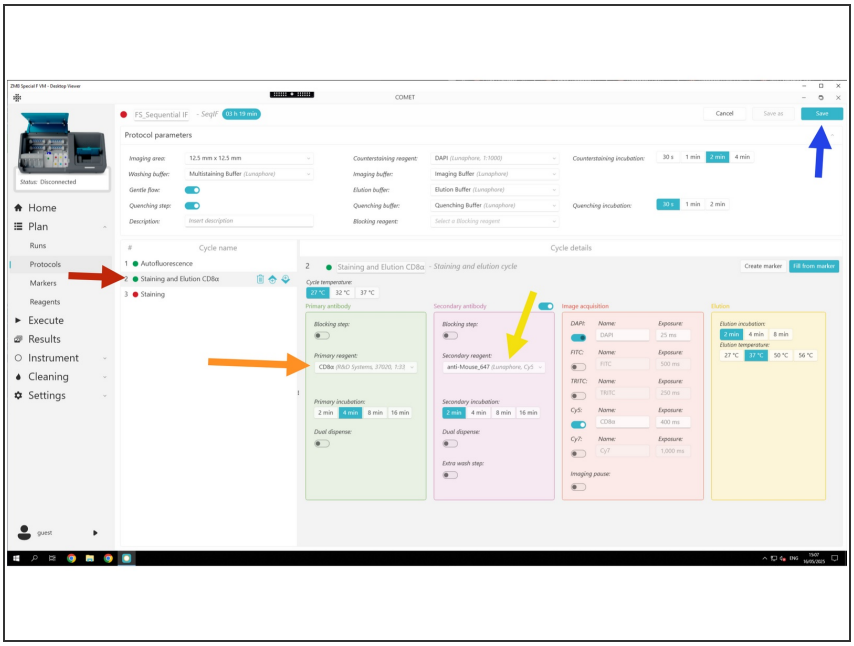
- FFPE – for paraffin-embedded samples
- FS – for fresh frozen samples

Step 8 — Editing Protocol Parameters



- Provide Name of your Protocol (**PI initials_Lab_User initials_Protocol**)
- Do not change default values (adapt as shown here).
  - Gentle Flow: Toggle ON to reduce flow pressure for delicate tissues (default for FS samples).
- Quenching is optional depending on your choice
- Blocking is optional depending on your choice
- Add AF to each channel name in order to label it as Autofluorescence channel for background subtraction

Step 9 — Editing Protocol Parameters and Imaging Settings



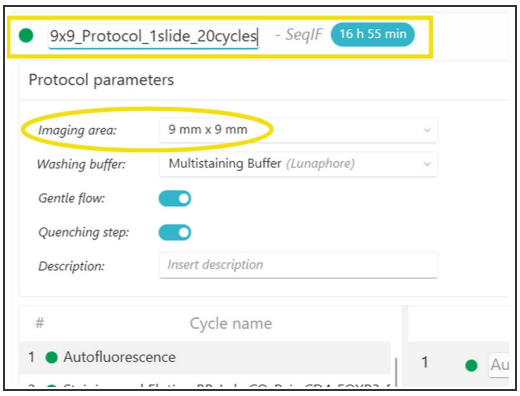
- Click **Staining and Elution** .
- Select your **primary antibody mix**.
- Select your **secondary antibody mix**.

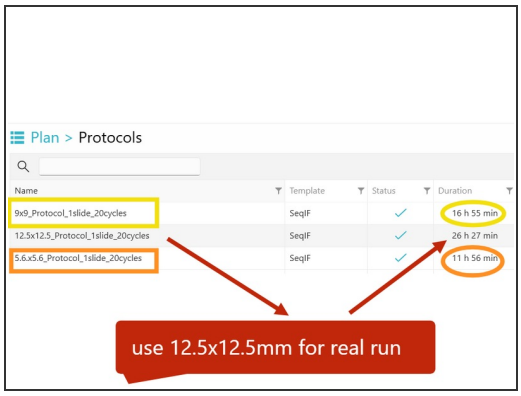
Keep all other settings as they are — the default values generally work well, unless you've already optimized them.

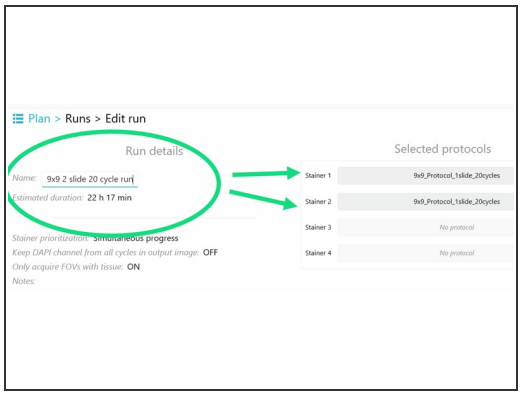
Do not change the exposure times for Alexa Fluor secondaries — they are pre-optimized by Lunaphore for proteomics. If you do adjust them, ensure they match the exposure times set in the autofluorescence acquisition tab.

- Once done with the protocol design click **Save**.

Step 10 — Mock Protocols & Runs for estimating Time based on Tissue Size within the Imaging Area







- **Take your protocol, 12.5mmx 12.5mm** (156.25mm2 =**100% tissue** coverage of imaging area) that you will use in the final run, and make a **mock 9mm x 9mm** (81mm2 = **50% tissue coverage** of imaging area) protocol. Save and use to design a Mock run for more accurate run time estimate.

⚠ **Note, for the Actual COMET Protocol and Run** use the 12.5x12.5 version, so that tissue can be detected throughout the entire imaging area, and not just a small 9x9 or 5.6x5.6 centered square within. The mock version is just to block the correct COMET run time in the booking calendar!

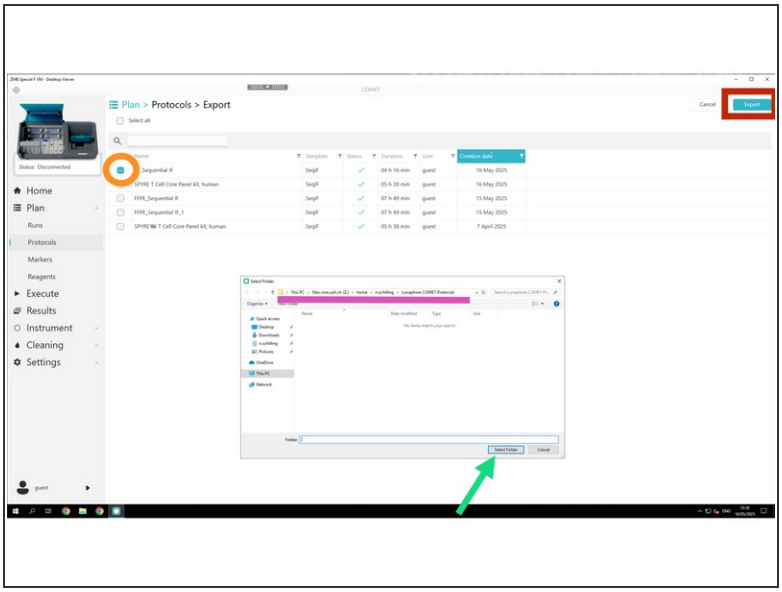
- For very small amounts of tissue instead take the same protocol and make a **mock 5.6mmx5.6mm** (31.36 mm2 =**20% of tissue coverage** in the imaging area)

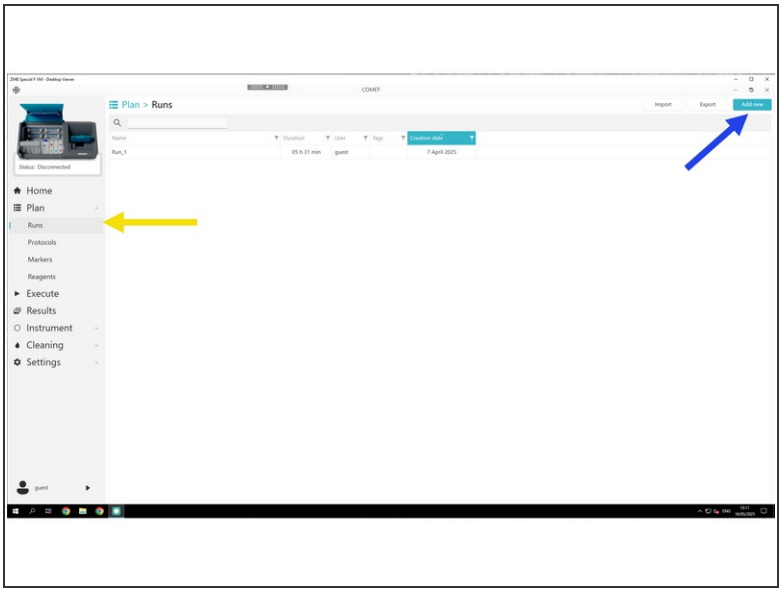
📌 Also use prior knowledge of previous run times for similar sized tissue with matching protocols, to better estimate booked COMET run time accurately. ZMB staff will not reduce overbooked hours on the COMET when users fail to accurately predict their run times.

- Comparison of example protocol times. **The same 20 cycle protocol** used for 1 slide results in: **100% Tissue Coverage of 12.5x12.5mm imaging area = 26hr 27min**. The same protocol on 1 slide **50% Tissue coverage 9x9mm imaging area = 16hr 55min**. The same on 1 slide with **20% Tissue coverage of 5.6x5.6mm imaging area = 11hr 56min**

- Comparison of example run times. **2 slides get parallelization**, faster run time than single slide total time. So **same 20 cycle protocol used for 2 slides results in: 100% Tissue Coverage of 12.5x12.5 = 33hr 31min**. The same on 2 slides with **50% Tissue coverage 9x9 = 22hr 17min**. 2 slides with **20% Tissue of 5.6x5.6 = 21 hrs**

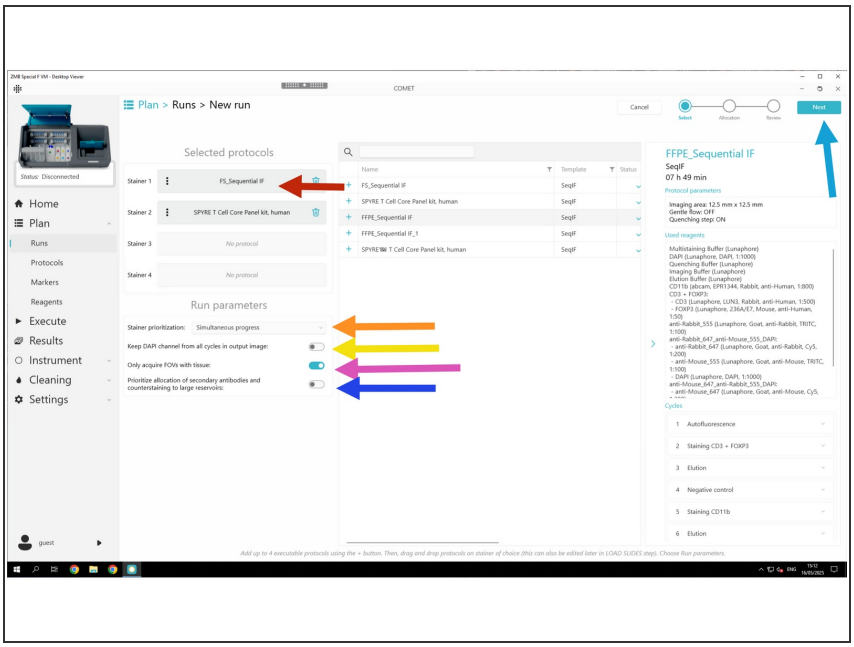
Step 11 — Export Protocols





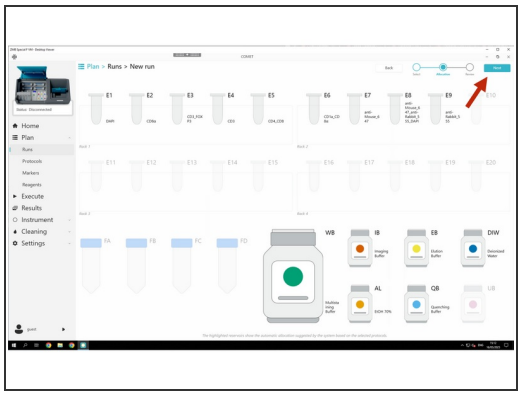
- Click **Export**
- Select the **Protocols** to be exported.
- Creat a new folder "Lunaphore COMET Protocols" in Z:\home\u.ser\Lunaphore COMET Protocols
- Export the selected protocols to Z:\home\u.ser\Lunaphore COMET Protocols
- Click **Run**
- Click **Add New**

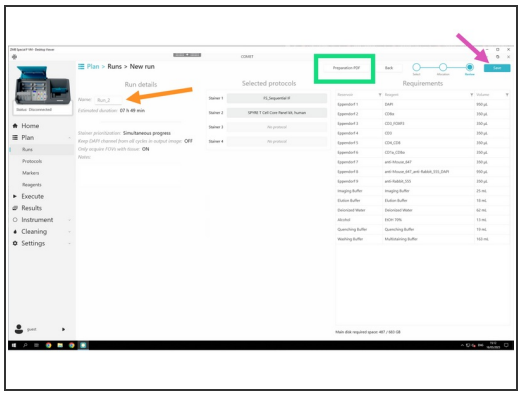
Step 12 — Create Run

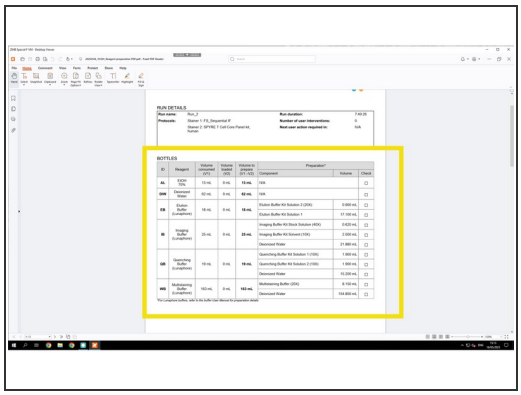


- Drag and drop the desired Protocols to the stainer of choice.
- Run all stainers simultaneously: enables parallel processing for higher throughput.
- Use same DAPI image for all cycles in the output image: **Disable** this to keep only the initial DAPI image.
- Enable Only acquire FOVs with tissue. Ensures imaging is restricted to tissue-containing areas.
- Prioritize allocation of secondary antibodies: **Disable** the fixed priority of secondaries in reservoir assignment — allows more flexible reagent allocation.
- Click **NEXT** to review settings.

Step 13 — Download Preparation PDF and export Run

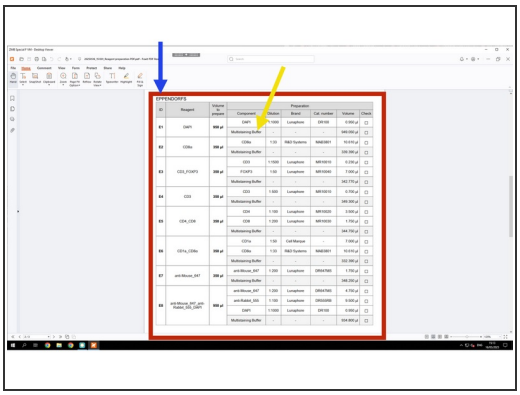


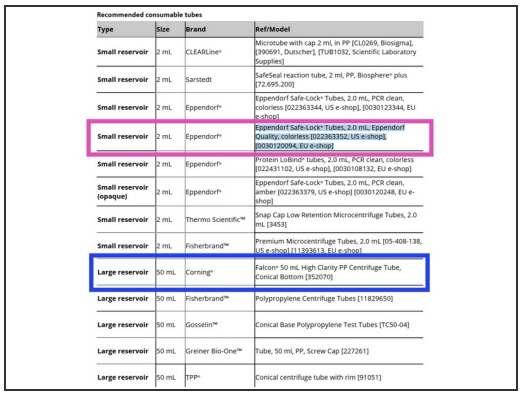




- Click **NEXT** to review settings.
- Follow the required naming convention: **PI initials_Lab_User initials_RUN_Name**
- Clicking **Preparation PDF** before saving lets you download the full reagent prep list.
- Click **Save** and save the RUN also under Z:\home\u.ser\Lunaphore COMET Run
- Since this is the Lunaphore COMET Emulator, it doesn't update buffer volumes from the actual COMET system in real time. Therefore, you can only know and prepare buffers during the actual hands-on training session.

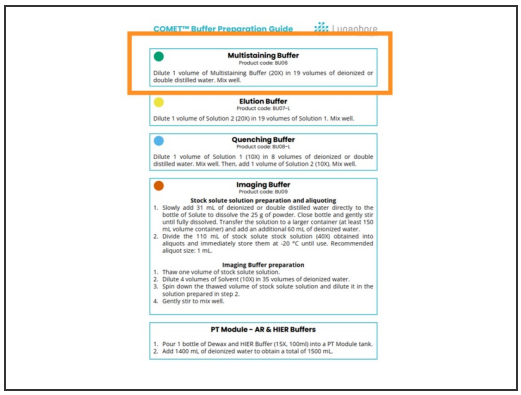
Step 14 — Prepare antibodies according to Preparation PDF





Recommended consumable tubes

Type	Size	Brand	Ref/Model
Small reservoir	2 mL	CLEARLine®	Microtube with cap 2 ml, in PP [CL0269, Biosigma], [390691, Dutscher], [TUB1032, Scientific Laboratory Supplies]
Small reservoir	2 mL	Sarstedt	SafeSeal reaction tube, 2 ml, PP, Biosphere® plus [72.695.200]
Small reservoir	2 mL	Eppendorf®	Eppendorf Safe-Lock® Tubes, 2.0 mL, PCR clean, colorless [022363344, US e-shop], [003012344, EU e-shop]
Small reservoir	2 mL	Eppendorf®	Eppendorf Safe-Lock® Tubes, 2.0 mL, Eppendorf Quality, colorless [022363352, US e-shop], [003012094, EU e-shop]
Small reservoir	2 mL	Eppendorf®	Protein LoBind® tubes, 2.0 mL, PCR clean, colorless [022431102, US e-shop], [003108132, EU e-shop]
Small reservoir (opaque)	2 mL	Eppendorf®	Eppendorf Safe-Lock® Tubes, 2.0 mL, PCR clean, amber [022363379, US e-shop] [003120248, EU e-shop]
Small reservoir	2 mL	Thermo Scientific™	Snap Cap Low Retention Microcentrifuge Tubes, 2.0 mL [3453]
Small reservoir	2 mL	Fisherbrand™	Premium Microcentrifuge Tubes, 2.0 mL [05-408-138, US e-shop] [11329613, EU e-shop]
Large reservoir	50 mL	Corning®	Falcon® 50 mL High Clarity PP Centrifuge Tube, Conical Bottom [352070]
Large reservoir	50 mL	Fisherbrand™	Polypropylene Centrifuge Tubes [11829650]
Large reservoir	50 mL	Gosselin™	Conical Base Polypropylene Test Tubes [TC50-04]
Large reservoir	50 mL	Greiner Bio-One™	Tube, 50 mL PP, Screw Cap [227261]
Large reservoir	50 mL	TPP®	Conical centrifuge tube with rim [91051]



COMET™ Buffer Preparation Guide — Lunaphore

Multistaining Buffer
Product code: BU09-L
Dilute 1 volume of Multistaining Buffer (20X) in 19 volumes of deionized or double distilled water. Mix well.

Elution Buffer
Product code: BU07-L
Dilute 1 volume of Solution 2 (20X) in 19 volumes of Solution 1. Mix well.

Quenching Buffer
Product code: BU08-L
Dilute 1 volume of Solution 1 (10X) in 8 volumes of deionized or double distilled water. Mix well. Then, add 1 volume of Solution 2 (10X). Mix well.

Imaging Buffer
Product code: BU06
Stock solute solution preparation and aliquoting
1. Slowly add 31 mL of deionized or double distilled water directly to the bottle of Solute to dissolve the 25 g of powder. Close bottle and gently stir until fully dissolved. Transfer the solution to a larger container (at least 150 mL volume container) and add an additional 60 mL of deionized water.
2. Divide the 110 mL of stock solute stock solution (40X) obtained into aliquots and immediately store them at -20 °C until use. Recommended aliquot size: 1 mL.

Imaging Buffer preparation
1. Thaw one volume of stock solute solution.
2. Dilute 4 volumes of Solvent (10X) in 35 volumes of deionized water.
3. Spin down the thawed volume of stock solute solution and dilute it in the solution prepared in step 2.
4. Gently stir to mix well.

PT Module – AR & HIER Buffers
1. Pour 1 bottle of Dewax and HIER Buffer (15X, 100ml) into a PT Module tank.
2. Add 1400 mL of deionized water to obtain a total of 1500 mL.

⚠ *The emulator helps save time during the actual COMET session by allowing you to pre-design protocols and prepare XML files. Based on the Preparation PDF, all antibodies must be pre-diluted and brought in 2 mL Eppendorf tubes only, which are required for use with the COMET.*

- Order for use on the system 2.0mL Eppendorf Safe-Lock Tubes Catalog#0030120094 Product # 700249 on P4U
- Dilute your antibodies according to the Preparation PDF.
  - Dilute in Multistaining Buffer (MSB), which is available in the COMET room. Arrange in advance with ZMB staff for an aliquot for your dilutions.

© 2026