Standard Operating Procedure

ProteinSimple Jess System for Automated Protein Analysis

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Equipment

- Jess™ instrument; ProteinSimple
- Centrifuge with plate adaptor
- Standard Heat Block Heater
- Vortex

Materials and Reagents

- Separation Module – Orange box (must be stored at 18-24 °C) with Capillary Cartridges, Pre-filled Microplates, 10X Sample Buffer, and Wash Buffer. Available separation modules with 13-Capillary Cartridge or 25-Capillary Cartridge (sold separately) for 12-230 kDa, 66-440 kDa and 2-40 kDa (all sold separately; note: most protein molecular weights range from 12-230 kDa).

- EZ Standard Pack (must be stored at 2-8 °C) including DTT, Fluorescence 5 Master Mix and Biotinylated Ladder.

- Protein samples (recombinant protein, over-expressed lysate, cell or tissue lysate) corresponding to the antibodies to be tested.

- Primary Antibodies

- Bio-Techne Anti-Rabbit or Anti-Mouse Detection Modules (respectively DM-001 and DM-002) containing Luminol-S (Cat. # 043-311), Peroxide (Cat. # 043-379), Streptavidin-HRP (Cat. # 042-414), Antibody Diluent #2 (Cat. # 042-203)

(Last Reviewed May 30th, 2025)

- (Optional) Bio-Techne RePlex Module (Cat. # RP-001), containing RePlex Reagent 1 (Cat. # RP-001-1) and RePlex Reagent 2 (RP-001-2)
- Water (molecular biology grade)
- Pipettes and Tips
- Microcentrifuge tubes
- Ice and ice bucket

Important Things to Consider

Before the Experiment

Plan the antibody multiplexing or RePlex well in advance. Some tips for antibody selection include the following:

- Chemiluminescence is used for low-expressed proteins. NIR (Alexa647) channel can be used for mid-high expressed proteins such as housekeeping genes or total proteins when performing phosphor-protein analysis. Reserve IR for highly abundant proteins since it is the channel with lower sensitivity
- When using highly specific antibodies, proteins with more than 30% difference in molecular weight can be tested in the same channel
- Avoid using primary antibodies produced in the same species as the target sample. That will reduce the event of crossreactivity with endogenous IgG, which is especially relevant for tissue lysates
- When running the optimization test, add appropriate controls to test the specificity of the secondary antibodies

During the Experiment

- Keep the lid on the assay plate, and don't remove the seal until you're ready to put the plate into the instrument. Keep the lid on between reagent additions and post-preparation
- If a row in the plate is used for the assay, fill all wells in that row. Don't run the assay with empty wells.

- Fill empty wells with the same volume of diluent used for the entire row or deionized water.

Separation Module Preparation

1. Refer to Separation Module – insert (SM-W001-SM-W0012) to mix Fluorescent 5X Master Mix, ladder and DTT.

2. Sample preparation

The optimal protein concentration depends on the expression level of your protein. Dilute lysate as necessary with 0.1X Sample Buffer. In general, the final concentration of protein should be 0.4 mg/mL for chemiluminescence or 1.0 mg/mL for fluorescence. It is recommended to try multiple sample concentrations or titrate the sample lysate. A good starting cell/tissue lysate concentration range to test is 2.00 mg/mL, 0.40 mg/mL, and 0.08 mg/mL. Pick the value that falls in the linear range.

Other sample-type concentrations

- Overexpressing cell lysate (0.25 mg/mL, 0.05 mg/ml, 0.01 mg/mL)
- Recombinant proteins (5 µg/mL, 1.0 µg/mL, 0.2 µg/mL)
- Dilute protein samples in 0.1X Sample Buffer (Dilute 10X Sample Buffer 1:100 in H2O) for an intermediate stock (IS) concentration.
- Combine 1 part 5X Fluorescent Master Mix with 4 parts IS protein preparation in a microcentrifuge tube to get the targeted final concentration. Mix with gentle pipetting. Example: Add 2 uL 5X Fluorescent Master Mix to 8 uL IS protein solution
- Denature samples and biotinylated ladder at 95°C, 5 min using the heat block heater. Then, do a quick spin at room temperature and store it on ice until ready to proceed with the microplate setup.

3. Antibody preparation

- Primary Antibody: Dilute in Antibody Diluent 2
    - Start with 3 screening dilutions. For most antibodies, test 1:10, 1:50, and 1:250 dilutions
      Or 100 ug/mL, 20 ug/mL, 4 ug/mL

- Alternatively, test 100x, 20x, 4x of your western blot antibody dilutions. For example, if your traditional western dilution is 1:2000, start with 1:20, 1:100, 1:500 in Simple Western
- Pick the concentration that falls into the saturation range

- Secondary (anti-mouse or anti-rabbit) It is provided ready to use in the Detection Module. If you're using your own, consult the Antibody Database on the ProteinSimple website (https://www.bio-techne.com/resources/simple-western-antibody-database)

4. Preparation of Luminol-S and Peroxide
    - Combine 200 uL of Luminol-S and 200 uL of Peroxide.
    - If running RePlex assay, a larger volume is needed, therefore combine 450 uL of Luminol-S and 450 uL of Peroxide. Dispense Luminol-S and Peroxide freshly prepared mix following RePlex Module (RP-001) insert. Note: Luminol-S/ Peroxide mixture should be prepared immediately before use.

5. Preparation of RePlex Reagents (Only if running RePlex assay)
    - Combine 1.4 mL of RePlex Reagent 1 and 0.35 mL of RePlex Reagent 2 in a microcentrifuge tube. Dispense following RePlex Module (RP-001) insert. Note: RePlex Reagents should be prepared immediately before use.

6. Pipetter your plate – refer to Separation Module inset (SM W001-SM W012), or RePlex™ Module insert RP-001 if running RePlex assay.

7. Centrifuge the plate for 5 minutes at 2500 rpm (~1000 x g) at room temperature with break set at 3. Ensure liquid is fully down in all wells and no bubbles are present.

8. Turn on the computer connected to Jess (the PC switch located behind the monitor). Login to the User account.

9. Turn on the instrument. The main power switch is located on the rear panel. Wait for Jess to initialize.

10. Double-click the Compass for Simple Western software icon to open the application.

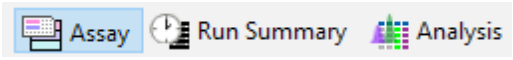
11. Connect to the Jess instrument.

12. Prepare an assay in the Compass software.

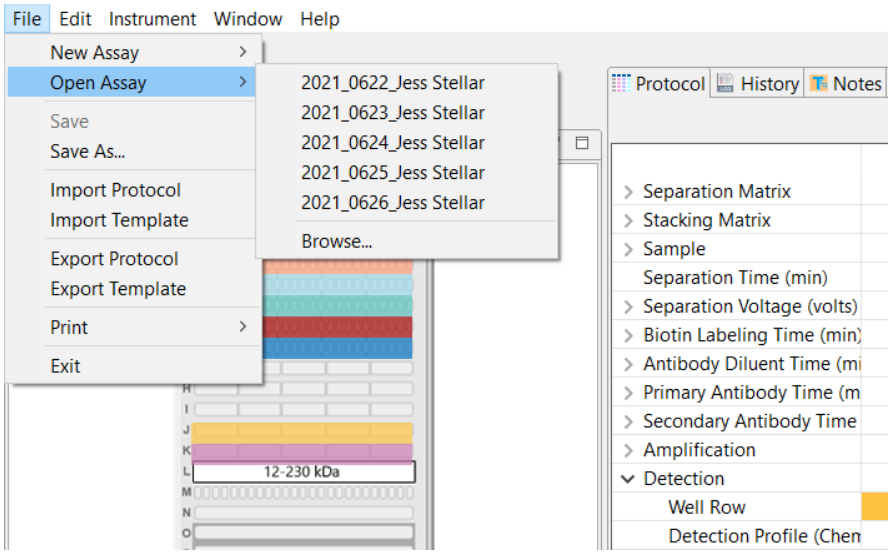
You can prepare an immunoassay in two ways, depending on whether you want to run an assay using existing parameters or set up a new one.

i. To start a new run with an existing assay:

a. Click on the Assay icon.



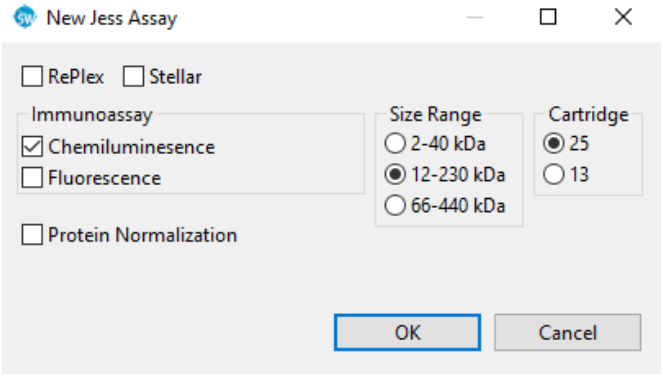
Select File in the main menu and click Open Assay.



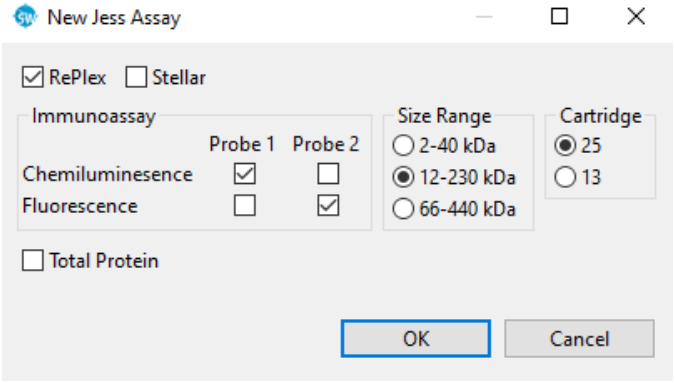
b. A list of the last five assays opened will be displayed. Select one of these assays or click Browse to open the Assay folder and select a different one.

ii. To start a run with a new assay, select File in the main menu and then click New Assay>Jess.

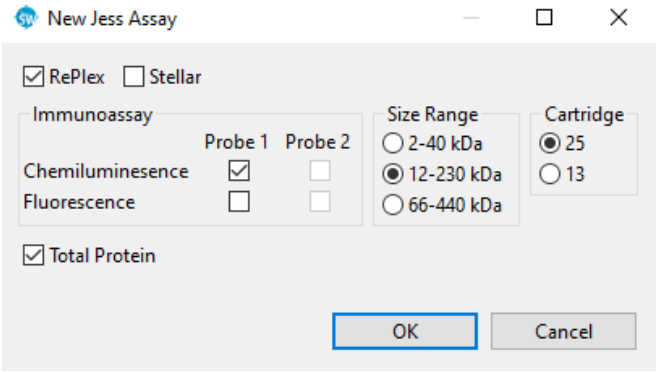
a. Standard Immunoassays - From the pop-up window, select the Assay Type (Chemiluminescence, Fluorescence and/or Protein Normalization). Select a Size Range (2-40 kDa, 12-230 kDa or 66-440 kDa) and a Cartridge (25 or 13 Capillary Cartridge). For example:



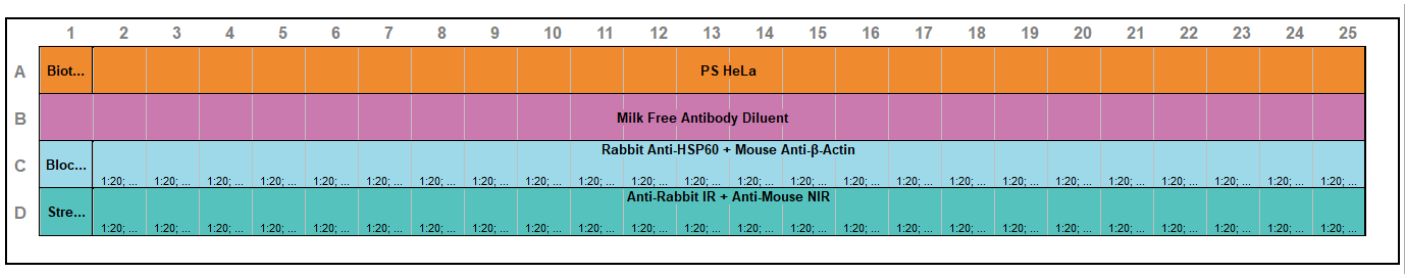
b. RePlex Assays:
To run two immunoassays - Select any combination of chemiluminescence or fluorescence for Probes 1 and 2. For example:



To run one immunoassay and one Total Protein assay - Select Total Protein, then select any combination of chemiluminescence or fluorescence for Probe 1. Probe 2 will be used for the Total Protein assay. For example:



i) Use the Assay tab to define the plate layout (sample position on the plate and concentration). See the template below as an example.



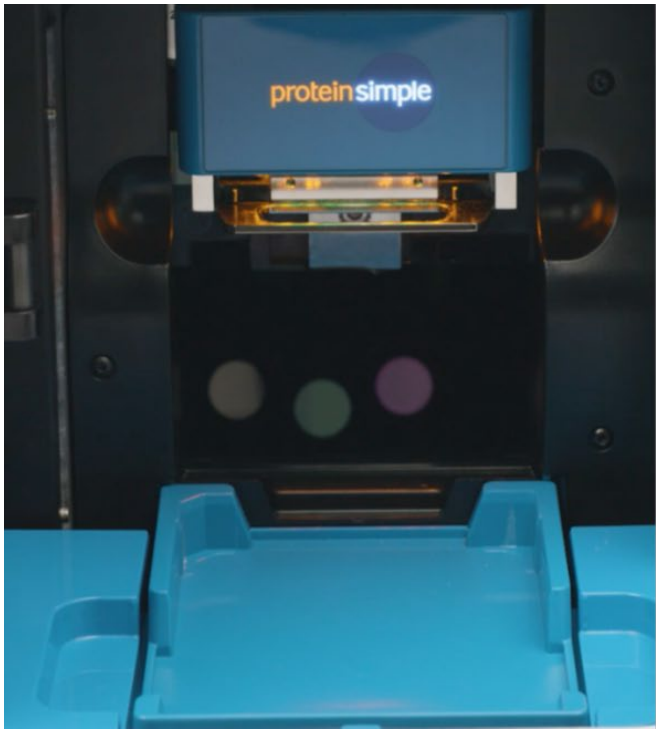
ii) When using the RePlex option, you must also fill in probe #2 information.

13. Open the instrument door. To open the door, make sure you see a steady blue LED on the front, then touch the small silver touchpad on Jess's door.

14. Insert a capillary cartridge into the cartridge holder. The interior light will change from orange to blue.

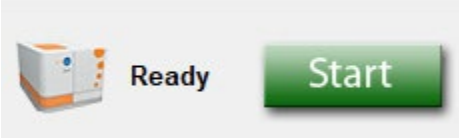
15. Remove the assay plate lid. Hold the plate firmly on bench and carefully peel off the evaporation seal. Pop any bubbles observed in the Separation Matrix wells with a pipette tip.

16. Place the assay plate on the plate holder. Sample plates must be inserted into the plate holder so that the A1 well position is aligned with the upper left corner of the tray.

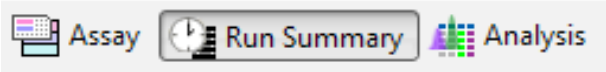


17. Close Jess door. To close, push the door until you feel Jess pull it closed.

18. Click the Start button in Compass.



To check the time remaining for your assay, click on the Run Summary icon



19. When the run is complete, discard the sample plate and cartridge. Dispose of used cartridges and plates as biohazard waste. Dispose of used cartridges in the sharps bin.

20. Create a new folder/subfolder in Documents to save data.

21. Shutdown the instrument
    - Make sure to remove any plates, cartridges and blots before shutting down the system.
    - Close Compass for Simple Western and shut down the computer.
    - The instrument should remain on unless you think it won't be used for over a week. In that case, you can just turn the power off.

Status Lights

The LED on Jess's front panel provides you with status updates:

• Start-up (magenta): You've turned on the power and the instrument is warming up.
• Ready (steady blue): Jess is powered on and ready for use.
• Opening Door (long blue flash followed by blue pulses): Jess's door is opening.
• Running (pulsing blue): Jess is running an assay.
• Trying to Open Door While Running (red flash): Jess can't open the door when she's running.
• Error (steady red): Jess has detected an error. To get more information, check the Status window of the Run Summary Screen in Compass for Simple Western.

Referenced Documents

- User Guide for Jess (https://resources.bio-techne.com/bio-techne-assets/docs/literature/Jess_User_Guide.pdf)
- Separation Module (SM-W001 to SM-W0012) (https://www.bio-techne.com/pdf-download-arena-document/product-insert/pl3-0005)
- RePlex Module (RP-001) (https://www.bio-techne.com/pdf-download-arena-document/product-insert/pl3-0029)
- Compass for Simple Western User Guide (https://resources.bio-techne.com/bio-techne-assets/docs/software/Simple%20Western/CompassforSW_7.0.0/Compass%20SW%20Software%20User%20Guide.pdf)