Proteograph® DIRECT Assay – Quick Reference

A Start the Instrument

1. Turn on SP200 Automation Instrument.
2. Turn on computer.
3. Launch Instrument Control Software (ICS).
4. Run Daily Maintenance.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	UC	
B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74		
C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	DDC	
D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	CC	
E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77		
F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78		
G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79		
H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80		

UC: User Control (supplied by user); DDC: Direct Digest Control;
CC: Cleanup Particles Control

B Prepare Reagents and Samples

1. Prepare Trypsin/LysC **on ice** by adding 500 µL of Enzyme Reconstitution Solution to each vial (final concentration: **0.2 µg/µL**).
2. Centrifuge samples at **5,000 g for 2 min at 4°C**.
3. Check labware for barcode labels.
4. Determine protein concentration (BCA recommended).
5. Dilute sample with TE buffer or PBS (final protein concentration: 0.025 – 1 µg/µL. Recommended 0.5 µg/µL)
6. Transfer **10 µL** sample to Preparation Plate.
7. Add **10 µL** user control to well A11.
   Note: *Do not add Direct Digest Control and Cleanup Particles Control at this step; they are added to the plate by the SP200 Automation Instrument during the assay run.*
8. Seal plate and centrifuge at **500 g for 15 sec**.
9. Hold the plate on ice until deck setup is complete (See "C: Run the Assay").

Continued on the other side.

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C Run the Assay

1. Verify correct labware placement in ICS.
2. Ensure the barcodes are visible and are facing **RIGHT**.
3. Unseal the Preparation Plate that was held on ice and place in Carrier **A4**.
4. Ensure carriers are pushed against stop hooks.
5. Close cover and start assay.

D Remove and Save the Plate

1. Unload carriers.
2. Remove collection plate and seal it.
3. Centrifuge at 500 g for 15 sec.
4. Set plate aside at room temperature and start Peptide Quantification within 1 hr.

Best Practices for Sample Handling

- The recommended lysis buffer for use with the Proteograph DIRECT workflow is 25 mM HEPES (pH 7.6) containing 0.5% Triton X-100.
  Using higher levels (>0.5 %) of detergent may increase risks of ion suppression in the mass spectrometer.
- For running replicates, transfer sample to a new tube after centrifugation, mix 5 – 10 times, then aliquot to the Preparation Plate.

Support

Send an email to **support@seer.bio**

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For research use only. Not for use in diagnostic procedures.

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