Related Consumables

INDIVIDUAL REAGENTS

PART #	ITEM
PS-ST01EZ-8	EZ Standard Pack 1 12-230 kDa
PS-ST02EZ-8	EZ Standard Pack 2 12-230 kDa
PS-ST03EZ-8	EZ Standard Pack 3 66-440 kDa
PS-ST04EZ-8	EZ Standard Pack 4 66-440 kDa
PS-ST05EZ-8	EZ Standard Pack 5 2-40 kDa
PS-FL01-8	Fluorescent 5x Master Mix 1
PS-FL03-8	Fluorescent 5x Master Mix 3
PS-FL05-8	Fluorescent 5x Master Mix 5
042-976	Total Protein Streptavidin-HRP
042-414	Streptavidin-HRP
043-816	Streptavidin-NIR
042-195	Sample Buffer
042-206	Anti-Rabbit Secondary HRP Antibody
043-819	Anti-Rabbit Secondary NIR Antibody
043-820	Anti-Rabbit Secondary IR Antibody
043-426	20X Anti-Rabbit HRP Conjugate

PART #	ITEM
042-205	Anti-Mouse Secondary HRP Antibody
043-821	Anti-Mouse Secondary NIR Antibody
043-822	Anti-Mouse Secondary IR Antibody
042-203	Antibody Diluent 2
042-196	10X System Control Primary Antibody-Rabbit for Chemiluminescence
042-191	10X System Control Primary Antibody-Mouse for Chemiluminescence
042-202	Wash Buffer
PS-CS01	Chemiluminescent Substrate
042-486	ERK1 Primary Antibody for Size Assays
042-488	HeLa Lysate Controls
043-311	Luminol-S
043-379	Peroxide
043-522-2	Anti-Goat Secondary HRP Antibody
043-491-2	Anti-Human IgG Secondary HRP Antibody
043-459-2	Secondary Streptavidin-HRP
043-524	Milk-free Antibody Diluent

ASSAY MODULES

PART #	ITEM
DM-001	Anti-Rabbit Detection Module
DM-002	Anti-Mouse Detection Module
DM-003	No secondary Detection Module
DM-004	Biotin Detection Module
DM-005	Anti-Human IgG Detection Module
DM-006	Anti-Goat Detection Module
DM-007	Anti-Rabbit NIR Detection Module
DM-008	Anti-Rabbit IR Detection Module
DM-009	Anti-Mouse NIR Detection Module
DM-010	Anti-Mouse IR Detection Module
DM-TP01	Total Protein Detection Module for Chemiluminescence based total protein assays
AM-PN01	Protein Normalization Assay Module for Fluorescence based total protein assays

PART #	ITEM
SM-W001	12-230 kDa Separation Module, 2 x 13 capillary cartridges
SM-W002	12-230 kDa Separation Module, 8 x 13 capillary cartridges
SM-W003	12-230 kDa Separation Module, 2 x 25 capillary cartridges
SM-W004	12-230 kDa Separation Module, 8 x 25 capillary cartridges
SM-W005	66-440 kDa Separation Module, 2 x 13 capillary cartridges
SM-W006	66-440 kDa Separation Module, 8 x 13 capillary cartridges
SM-W007	66-440 kDa Separation Module, 2 x 25 capillary cartridges
SM-W008	66-440 kDa Separation Module, 8 x 25 capillary cartridges
SM-W009	2-40 kDa Separation Module, 2 x 13 capillary cartridges
SM-W010	2-40 kDa Separation Module, 8 x 13 capillary cartridges
SM-W011	2-40 kDa Separation Module, 2 x 25 capillary cartridges
SM-W012	2-40 kDa Separation Module, 8 x 25 capillary cartridges

CONSUMABLES

PART #	ITEM
PS-PP03	12-230 kDa Pre-filled Plates
PS-PP04	66-440 kDa Pre-filled Plates
PS-PP05	2-40 kDa Pre-filled Plates

PART #	ITEM
PS-CC01	25-Capillary cartridges for Size based Separation
PS-CC02	13-Capillary cartridges for Size based Separation

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a bio-techne brand

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PL3-0015 Rev C

Protein Normalization Assay Module for Fluorescence Based Total Protein Assays

Jess
AM-PN01

Let's get started!

Reagents and materials

ORANGE BOX — STORE AT 18–24 °C

INCLUDES	PART NO
25-Capillary Cartridge (8 pack) 12–230 kDa Pre-filled Microplates with Split Running Buffer (8) Wash Buffer (60 mL) 10X Sample Buffer (440 µL)	SM-PN01-1

CLAMSHELL 1 — STORE AT 2–8 °C

INCLUDES	PART NO
EZ Standard Pack (8 pack) Ready-to-use Biotinylated Ladder 12–230 kDa Fluorescent 5X Master Mix DTT	PS-ST01EZ-8

CLAMSHELL 2 — STORE AT -70 TO -90 °C

INCLUDES	PART NO
Protein Normalization Reagent (8 tubes)	043-824

CLAMSHELL 3 — STORE AT 18–24 °C

INCLUDES	PART NO
Protein Normalization Reconstitution Agent (3.5 mL)	043-823

Other things you'll need

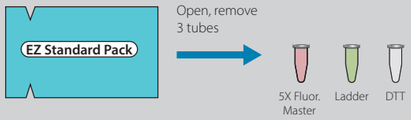
- Protein samples
- Primary antibodies
- Water, 0.22 µm-filtered and deionized (molecular biology grade or better)
- Pipettes and tips
- Microcentrifuge and tubes
- Ice and ice bucket
- Vortex
- Heat block
- Centrifuge with plate adapter

A few things you should know

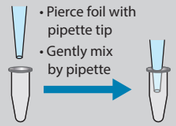
- Only use the provided capillary cartridges with the Protein Normalization reagent.
- Warm microplates up to room temperature for at least 24 hours before you start the first assay.
- Capillaries are moisture- and light-sensitive.
- Store unopened cartridge packages and plates at room temperature and do not remove the seals until ready to use.
- The first capillary in the cartridge has been optimized for running the ready-to-use biotinylated ladder. Pipette the biotinylated ladder and samples only as shown in Step 3.
- Evaporation of wells in a plate dramatically affects experimental results. To prevent evaporation, keep the lid on the assay plate and do not remove the evaporation seal until you're ready to put the assay plate into the instrument. Keep the lid on between reagent additions and post-preparation.
- An optional System Control Primary Antibody (PN 042-196 or 042-191) can be mixed with your primary antibody in the assay to calculate inter-assay and inter-instrument variability (for chemiluminescence only).
- You can use Bicine/CHAPS buffer (PN 040-764) or RIPA buffer (PN 040-483) to lyse your cells.

1. Assay Module preparation

A PREPARE STANDARD PACK REAGENTS

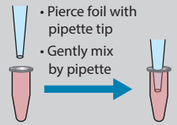


DTT (Clear Tube)



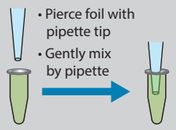
- Add 40 µL deionized water to make a 400 mM solution

Fluorescent 5X Master Mix (Pink Tube)



- Add 20 µL 10X Sample Buffer
- Add 20 µL prepared 400 mM DTT solution

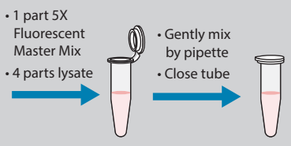
Biotinylated Ladder (Green Tube with Pink Pellet)



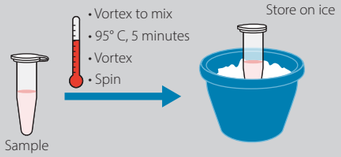
- Add 20 µL deionized water

B PREPARE YOUR SAMPLES

- The optimal protein concentration depends on the expression level of your protein. Dilute lysate as necessary with 0.1X Sample Buffer.
- Combine 1 part 5X Fluorescent Master Mix with 4 parts diluted lysate in a microcentrifuge tube (final concentration 0.4 mg/mL for chemiluminescence or 1.0 mg/mL for fluorescence). Produce enough diluted sample volume required for assay.

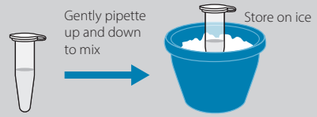


C DENATURE YOUR SAMPLES



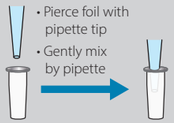
D MIX LUMINOL-S AND PEROXIDE (IF APPLICABLE)

- Combine 200 µL Luminol-S and 200 µL Peroxide in a microcentrifuge tube



E PREPARE PROTEIN NORMALIZATION REAGENTS

- The Protein Normalization Reagent should only be prepared immediately before loading the plate.



- Prepare the Protein Normalization Reagent stock solution by adding 100 µL Protein Normalization Reconstitution Agent per tube. Thoroughly mix the reagent by pipetting 15 times.
- Use the following table to prepare the working solution of the reconstituted Protein Normalization Reagent stock solution. Thoroughly mix the working solution by pipetting 15 times.

PROTEIN MOLECULAR WEIGHT RANGE		
LYSATE CONCENTRATION	12–230 kDa	
	Stock Solution	Reconstitution Agent
0.2–1.2 mg/mL	50 µL	250 µL

2. Detection Module preparation

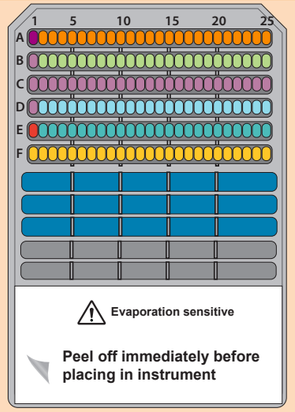
FOR CHEMILUMINESCENCE DETECTION

- Refer to Chemiluminescence Detection Module insert for instructions on primary and secondary antibody preparations

FOR FLUORESCENCE DETECTION

- Refer to Fluorescence Detection Module insert for instructions on primary and secondary antibody preparations

3. Pipette your plate



Biotinylated Ladder, 5 µL; Prepared Samples, 3 µL
Milk-Free Antibody Diluent, 10 µL; Protein Normalization Reagent, 10 µL
Milk-Free Antibody Diluent, 10 µL
Milk-Free Antibody Diluent, 10 µL; Primary Antibody, 10 µL
Streptavidin-HRP or -NIR, 10 µL; Secondary Antibody, 10 µL
Luminol-Peroxide Mix, 15 µL (if applicable)

Wash Buffer
500 µL/compartment

For more consistent results, keep the lid on the microplate between reagent additions and minimize bubble formation when adding Wash Buffer to the troughs and microplates. Protein Normalization reagent should be dispensed last.

1. Dispense reagents into the assay plate using the volumes shown in the plate diagram.
2. Centrifuge the plate for 5 minutes at 2500 rpm (~1000 x *g*) at room temperature. Ensure liquid is fully down in all wells.

4. Start Jess

1. Select the desired assay parameters in Compass software.
2. Open Jess's door.
3. Insert a capillary cartridge into the cartridge holder. The interior light will change from orange to blue.
4. Remove the assay plate lid. Hold plate firmly on bench and carefully peel off evaporation seal. Pop any bubbles observed in the Separation Matrix wells with a pipette tip.
5. Place the assay plate on the plate holder.
6. Close Jess's door.
7. Click the Start button in Compass.
8. When the run is complete, discard the plate and cartridge.