Fluorescence and Chemiluminescence Training Kit

PS-T005

Let's get started!

Reagents and materials

ORANGE BOX — STORE AT 18–28 °C

INCLUDES	PART NO
Wash Buffer (65 mL)	042-202
10X Sample Buffer (440 µL)	042-195
12-230 kDa Pre-Filled Microplates (8)	043-165
Capillary Cartridges – Fluorescence (8)	009-401

CLAMSHELL 1 — STORE AT 2–8 °C

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

CLAMSHELL 2 — STORE AT 2–8 °C

INCLUDES	PART NO
Fluorescence Reagents	
Anti-Rabbit Secondary NIR Antibody (130 uL)	043-819
Anti-Rabbit Secondary IR Antibody (130 uL)	043-820
Anti-Mouse Secondary NIR Antibody (130 uL)	043-821
Anti-Mouse Secondary IR Antibody (130 uL)	043-822
Milk-Free Antibody Diluent (20 mL)	043-524
Streptavidin-NIR (96 uL)	043-816
Reconstitution Reagent 2 (0.5 mL)	106-0006

CLAMSHELL 3 — STORE AT -70 TO -90 °C

INCLUDES	PART NO
HeLa Lysate Control for Size (2 x 20 µl)	042-488

CLAMSHELL 4 — STORE AT -10 TO -30 °C

INCLUDES	PART NO
β-Actin Primary Antibody – Mouse	MAB8929
HSP60 Primary Antibody – Rabbit	AF1800

CLAMSHELL 5 — STORE AT 2–8 °C

INCLUDES	PART NO
Chemiluminescence Reagents	
Anti-Rabbit Secondary Antibody (2 mL)	042-206
Luminol (2 mL)	043-311
Peroxide (2 mL)	043-379
Antibody Diluent 2 (20 mL)	042-203
Streptavidin-HRP (132 µL)	042-414

CLAMSHELL 6 — STORE AT 2–8 °C

INCLUDES	PART NO
10X System Control Primary Antibody - Rabbit (250 µL)	042-196
ERK1 Primary Antibody – Rabbit (0.5 mL)	042-486
Anti-Mouse Secondary Antibody (2 mL)	042-205

Other things you'll need

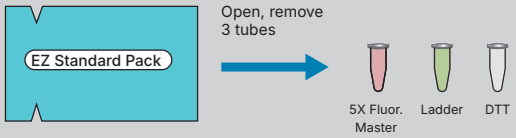
- Water, 0.22 µm-filtered and deionized (molecular biology grade or better)
- Pipettes and tips, ideally a repeater pipette
- Microcentrifuge and tubes
- Ice and ice bucket
- Vortex
- Heat block or PCR machine
- Centrifuge with plate adapter

A few things you should know

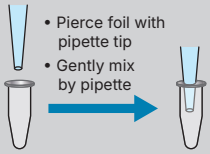
- Warm microplates up to room temperature for at least 24 hours before you start the first assay.
- Capillaries are moisture- and light-sensitive.
- Store unopened cartridge packages and plates at room temperature and do not remove the seals until ready to use.
- The first capillary in the cartridge has been optimized for running the ready-to-use biotinylated ladder. Pipette the biotinylated ladder and samples only as shown in Step 3.
- Plate well evaporation 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.
- The Biotinylated Ladder, Milk-Free Antibody Diluent, Antibody Diluent 2, Sample Buffer (10X), Streptavidin-NIR and -HRP, and Wash Buffer are ready-to-use reagents and should not be diluted.
- Chemiluminescence only: An optional System Control Primary Antibody (042-196 or 042-191) can be mixed with your primary antibody in the assay to calculate inter-assay and inter-instrument variability.

1. Reagent and sample preparation

A PREPARE STANDARD PACK REAGENTS

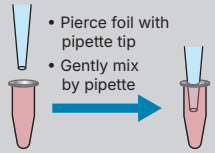


DTT (Clear Tube)



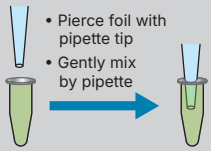
- Add 40 µL deionized water to make a 400 mM solution

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)

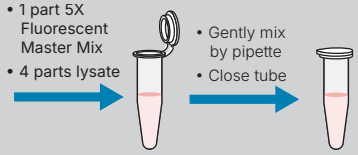


- Add 20 µL deionized water

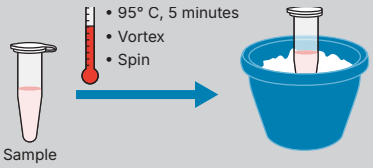
For Fluorescence Runs:

B PREPARE HELA LYSATE SAMPLE

- Prepare 0.1X Sample Buffer by mixing:
  - 1.5 µL 10X Sample Buffer
  - 148.5 µL deionized water
- Dilute the HeLa Lysate Control by mixing:
  - 18 µL stock Hela Lysate
  - 72 µL of 0.1X Sample Buffer
- Combine 20 µL 5X Fluorescent Master Mix with 80 µL diluted HeLa lyaste in a microcentrifuge tube.



C DENATURE YOUR SAMPLES

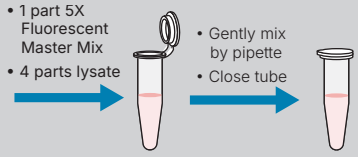


1. Reagent and sample preparation continued

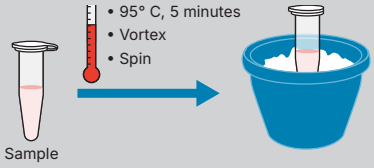
For Chemiluminescence Runs:

B PREPARE YOUR SAMPLES

- Prepare 0.1X Sample Buffer by mixing:
  - 1.5 µL 10X Sample Buffer
  - 148.5 µL deionized water
- Dilute the HeLa Lysate Control by mixing:
  - 12.5 µL stock HeLa lysate
  - 112.5 µL of 0.1X Sample Buffer
- Combine 20 µL 5X Fluorescent Master Mix with 80 µL diluted HeLa lyaste in a microcentrifuge tube.



C DENATURE YOUR SAMPLES



D MIX LUMINOL-S AND PEROXIDE

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

2. Antibody preparation

FOR FLUORESCENCE RUNS: PRIMARY ANTIBODY MULTIPLEX MIX

- Add 125 µL Reconstitution Reagent 2 to HSP60 Primary Antibody, and gently mix by pipette
- Add 200 µL Reconstitution Reagent 2 to β-Actin Primary Antibody, and gently mix by pipette
- Combine the following to prepare Primary Antibody Multiplexing Mix:
  - HSP60 Antibody – 15 µL
  - β-Actin Antibody – 6 µL
  - Milk-Free Antibody Diluent – 279 µL

SECONDARY ANTIBODY MULTIPLEX MIX

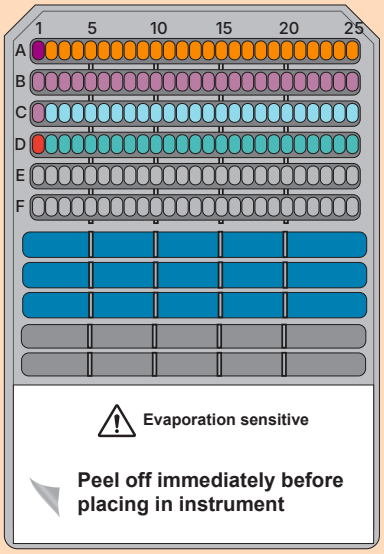
- Combine the following to prepare Secondary Antibody Multiplexing Mix:
  - Anti-Rabbit Secondary IR Antibody – 15 µL
  - Anti-Mouse Secondary NIR Antibody – 15 µL
  - Milk-Free Antibody Diluent – 270 µL

FOR CHEMILUMINESCENCE RUNS: PREPARE YOUR ANTIBODIES

- Primary Antibody: The Rabbit Anti-ERK1 Primary Antibody is ready to use. Optional: If you're using the System Control, add 25 µL of the System Control Primary Antibody to 225 µL of the ERK1 Primary Antibody. Pipette 10 µL of the mixture into each Primary Antibody well.
- Secondary Antibodies: The supplied antibodies are ready to use without dilution. Use the Anti-Rabbit Secondary Antibody with the anti-ERK1 antibody.

3. Pipette your plate

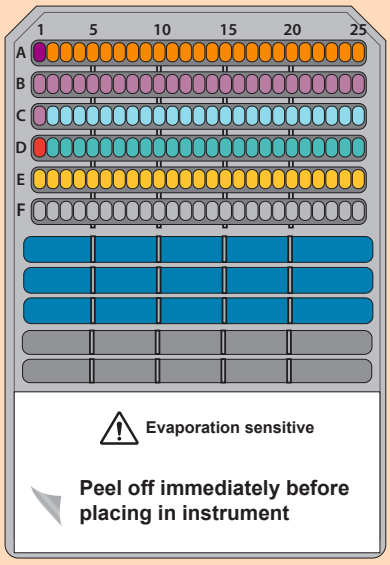
FOR FLUORESCENCE RUNS:



Biotinylated Ladder, 5 µL; HeLa Lysate, 3 µL
Antibody Diluent (2 or Milk-Free), 10 µL
Antibody Diluent (2 or Milk-Free), 10 µL; Primary Antibody Mix, 10 µL
Streptavidin-NIR, 10 µL; Secondary Antibody Mix, 10 µL

Wash Buffer
500 µL/compartment

FOR CHEMILUMINESCENCE RUNS:



Biotinylated Ladder, 5 µL; HeLa Lysate, 3 µL
Antibody Diluent 2, 10 µL
Antibody Diluent 2, 10 µL; Rabbit Anti-ERK1 Primary Antibody, 10 µL
Streptavidin-HRP, 10 µL; Anti-Rabbit Secondary Antibody, 10 µL
Luminol-Peroxide Mix, 15 µL

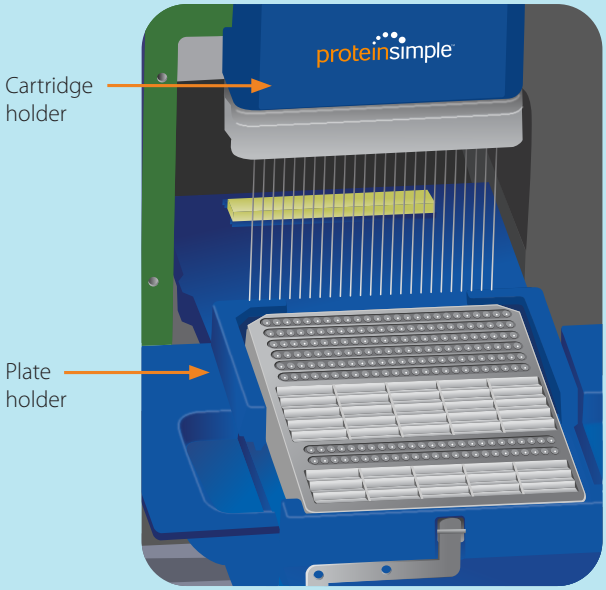
Wash Buffer
500 µL/compartment

For more consistent results, keep the lid on the microplate between reagent additions and minimize bubble formation when adding Wash Buffer to the troughs.

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 assay parameters in Compass software. New Assay>Jess–Immunoassay: Fluorescence or Chemiluminescence; Size Range:12-230 kDa; Cartridge: 25.
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.



Related Consumables

INDIVIDUAL REAGENTS

PART NO	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 NO	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 NO	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
DM-PN02	Protein Normalization Module for Fluorescence based total protein assays
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

PART NO	ITEM
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, 2 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
SM-FL001	12-230 kDa Fluorescence Separation Module, 8 x 13 capillary cartridges
SM-FL002	66-440 kDa Fluorescence Separation Module, 8 x 13 capillary cartridges
SM-FL003	2-40 kDa Fluorescence Separation Module, 8 x 13 capillary cartridges
SM-FL004	12-230 kDa Fluorescence Separation Module, 8 x 25 capillary cartridges
SM-FL005	66-440 kDa Fluorescence Separation Module, 8 x 25 capillary cartridges
SM-FL006	2-40 kDa Fluorescence Separation Module, 8 x 25 capillary cartridges

CONSUMABLES

PART NO	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 NO	ITEM
PS-CC01	25-Capillary cartridges for Size based Separation
PS-CC02	13-Capillary cartridges for Size based Separation

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PL3-0043 Rev C