

# Related Consumables

## INDIVIDUAL REAGENTS

PART #	ITEM
PS-ST01-EZ-8	EZ Standard Pack 1 12-230 kDa
PS-ST02-EZ-8	EZ Standard Pack 2 12-230 kDa
PS-ST03-EZ-8	EZ Standard Pack 3 66-440 kDa
PS-ST04-EZ-8	EZ Standard Pack 4 66-440 kDa
PS-ST05-EZ-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	Jess, Wes, Sally Sue, Peggy Sue 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	Erk Primary Antibody for Size Assays
042-488	HeLa Lysate Controls for Jess, Wes, Sally Sue and Peggy Sue
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 HRP Detection Module for Jess, Wes, Peggy Sue or Sally Sue
DM-002	Anti-Mouse HRP Detection Module for Jess, Wes, Peggy Sue or Sally Sue
DM-003	No secondary Detection Module for Jess, Wes, Peggy Sue or Sally Sue
DM-004	Biotin Detection Module for Jess, Wes, Peggy Sue or Sally Sue
DM-005	Anti-Human IgG HRP Detection Module for Jess, Wes, Peggy Sue or Sally Sue
DM-006	Anti-Goat HRP Detection Module for Jess, Wes, Peggy Sue or Sally Sue
DM-007	Anti-Rabbit NIR Detection Module for Jess
DM-008	Anti-Rabbit IR Detection Module for Jess
DM-009	Anti-Mouse NIR Detection Module for Jess
DM-010	Anti-Mouse IR Detection Module for Jess
DM-TP01	Total Protein Detection Module for Jess, Wes, Peggy Sue or Sally Sue
DM-TP02	Protein Normalization Module for Jess

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

## CONSUMABLES

PART #	ITEM
PS-PP03	Jess/Wes 12-230 kDa Pre-filled Plates with Split Buffer
PS-PP04	Jess/Wes 66-440 kDa Pre-filled Plates with Split Buffer
PS-PP05	Jess/Wes 2-40 kDa Pre-filled Plates with Split Buffer

PART #	ITEM
PS-CC01	Jess/Wes 25-Capillary Cartridges (8 pack)
PS-CC02	Jess/Wes 13-Capillary Cartridges (8 pack)



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PL3-0006 RevA



## Let's get started!

### Reagents and materials

#### ORANGE BOX — STORE AT 18–24 °C

INCLUDES	PART NO
Wash Buffer (60 mL)	042-202
10X Sample Buffer (440 µL)	042-195
12–230 kDa Pre-Filled Microplates (8)	043-165
Capillary Cartridges (8)	009-050

#### CLAMSHELL 1 — STORE AT 2–8 °C

INCLUDES	PART NO
Ready-to-use Biotinylated Ladder (12–230 kDa)	PS-ST01-EZ-8
Fluorescent 5X Master Mix	
DTT	

#### CLAMSHELL 2 — STORE AT 2–8 °C

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

#### CLAMSHELL 3 — STORE AT -80 °C

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

#### CLAMSHELL 4 — STORE AT -20 °C

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

### Other things you'll need

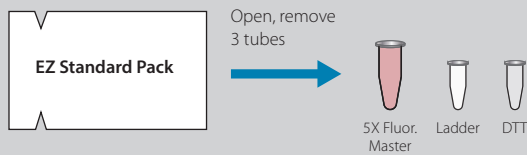
- Water, 0.22 µm-filtered and deionized (molecular biology grade or better)
- Pipettes and tips, ideally a repeater pipette
- Microcentrifuge and tubes
- Ice and ice bucket
- Vortex
- Heat block or PCR machine
- Centrifuge with plate adapter

### A few things you should know

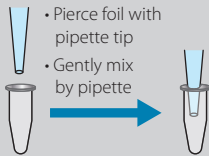
- Warm microplates up to room temperature for at least 24 hours before you start the first assay.
- Capillaries are moisture- and light-sensitive.
- Store unopened cartridge packages and plates at room temperature and do not remove the seals until ready to use.
- The first capillary in the cartridge 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, Sample Buffer (10X), Streptavidin-NIR, and Wash Buffer are ready-to-use reagents and should not be diluted.

# 1. Reagent and sample preparation

## A PREPARE STANDARD PACK REAGENTS

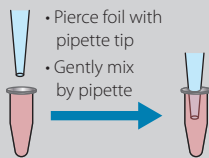


### DTT (Clear Tube)



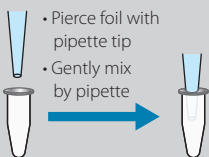
- Add 40  $\mu\text{L}$  deionized water to make a 400 mM solution

### Fluorescent 5X Master Mix (Pink Tube)



- Add 20  $\mu\text{L}$  10X Sample Buffer
- Add 20  $\mu\text{L}$  prepared 400 mM DTT solution

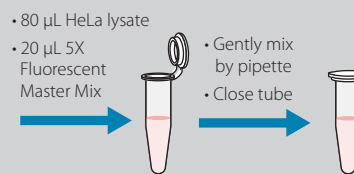
### Biotinylated Ladder (Clear Tube with Pink Pellet)



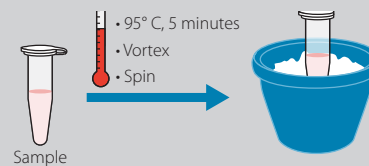
- Add 20  $\mu\text{L}$  deionized water

## B PREPARE HELA LYSATE SAMPLE

1. Prepare 0.1X Sample Buffer by mixing:
  - 1.5  $\mu\text{L}$  10X Sample Buffer
  - 148.5  $\mu\text{L}$  deionized water
2. Dilute the HeLa Lysate Control by mixing:
  - 18  $\mu\text{L}$  stock HeLa Lysate
  - 72  $\mu\text{L}$  of 0.1X Sample Buffer
3. Combine 20  $\mu\text{L}$  5X Fluorescent Master Mix with 80  $\mu\text{L}$  diluted HeLa lysate



## C DENATURE YOUR SAMPLES



# 2. Antibody preparation

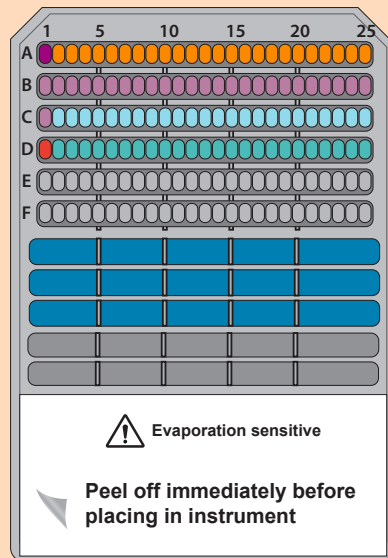
## PRIMARY ANTIBODY MULTIPLEX MIX

- Add 125  $\mu\text{L}$  Reconstitution Reagent 2 to HSP60 Primary Antibody, and gently mix by pipette
- Add 200  $\mu\text{L}$  Reconstitution Reagent 2 to  $\beta$ -Actin Primary Antibody, and gently mix by pipette
- Combine the following to prepare Primary Antibody Multiplexing Mix:
  - HSP60 Antibody – 15  $\mu\text{L}$
  - $\beta$ -Actin Antibody – 6  $\mu\text{L}$
  - Milk-Free Antibody Diluent – 279  $\mu\text{L}$

## SECONDARY ANTIBODY MULTIPLEX MIX

- Combine the following to prepare Secondary Antibody Multiplexing Mix:
  - Anti-Rabbit Secondary IR Antibody – 15  $\mu\text{L}$
  - Anti-Mouse Secondary NIR Antibody – 15  $\mu\text{L}$
  - Milk-Free Antibody Diluent – 270  $\mu\text{L}$

## 3. Pipette your plate



- Biotinylated Ladder, 5  $\mu$ L; ● Prepared Samples, 3  $\mu$ L
  - Milk-Free Antibody Diluent, 10  $\mu$ L
  - Milk-Free Antibody Diluent, 10  $\mu$ L; ● Primary Antibody Multiplex Mix, 10  $\mu$ L
  - Streptavidin-NIR, 10  $\mu$ L; ● Secondary Antibody Multiplex Mix, 10  $\mu$ L
- Wash Buffer  
● 500  $\mu$ L/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 ( $\sim$ 1000  $\times$   $g$ ) 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; 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.

