MSD® Multiplexed Phosphoprotein Assay: Total and Phospho-c-Kit Duplex

MULTI-SPOT® 4, 96-well Custom Plate

MSD Materials

	Storage
❑ Read Buffer T (with surfactant), 4X	RT
❑ MULTI-SPOT 4, 96-well Custom plates	4ºC
❑ Tris Wash Buffer (10X)	4ºC
❑ Tris Lysis Buffer (1X)	4ºC
❑ Phosphatase inhibitor I (100X)	4ºC
❑ Phosphatase inhibitor II (100X)	4ºC
❑ Protease inhibitor cocktail (50X)	-20ºC
❑ SULFO-TAG™ detection antibody	4ºC
❑ Blocker A	4ºC

Other Materials & Equipment (not supplied)

- ❑ Deionized water for diluting Wash Buffer and Read Buffer
- ❑ One 250 mL bottle
- ❑ Two 50 mL tube
- ❑ One 15 mL tube
- ❑ Various microcentrifuge tubes for making serial dilutions of lysates (if desired)
- ❑ Automated plate washer, Multidrop®, or other efficient multi-channel pipetting equipment for washing 96 well plates
- ❑ Appropriate liquid handling equipment for desired throughput that must accurately dispense 25 µL and 150 µL into a 96-well micro plate

Protocol at a Glance

Notes:

Read the entire detailed instructions before beginning work.

The following protocol describes the most conservative approach toward achieving highly sensitive results using MSD technology to quantify phosphoproteins. The protocol takes approximately 3 to 3½ hours to complete if each reagent is prepared during the preceding incubation. All reagents with the exception of diluted lysates can also be prepared ahead of time. This lengthens the overall time required for the assay but frees up time during incubation steps.

Once desired results are achieved, the protocol can be streamlined to eliminate multiple incubation and wash steps to increase throughput.

1. Add blocking solution, incubate 1 hour, wash.
2. Add sample or lysate, incubate 1 hour, wash.
3. Add detection antibody, incubate 1 hour, wash.
4. Add Read Buffer and analyze plate.

Detailed Instructions

A larger amount of Wash Buffer may be prepared and stored at room temperature for later use.

Prepare a stock of 1X Tris Wash Buffer:

a) 1X Tris Wash Buffer will be used throughout the assay to make other reagents as well as wash plates. Approximately 250 mL per plate is required– more if using an automatic plate washer.

b) In a 250 mL bottle combine:
   - ❑ 25 mL 10X Tris Wash Buffer
   - ❑ 225 mL deionized water

Prepare Blocking Solution-A:

a) Prepare 20 mL per plate.

b) In a 50 mL tube combine:
   - ❑ 20 mL 1X Tris Wash Buffer
   - ❑ 600 mg Blocker A (30 mg/mL or 3%)

Solutions containing Blocker A should be kept at 4ºC and discarded after 14 days.

Prepare Antibody Dilution Buffer:

a) Prepare 3 mL per plate.

b) In a 15 mL tube combine:
   - ❑ 1 mL Blocking Solution-A
   - ❑ 2 mL 1X Tris Wash Solution

Begin with a MULTI-SPOT Custom Plate. No pre-treatment is necessary.

Save the plate packaging or copy the diagram of the capture antibody array into your notebook. Data will be labeled according to the location of each spot, not the actual name of the coating.

STEP 1

Add 150 µL/well of Blocking Solution-A.

Incubate with shaking at room temperature for 1 hour. Prepare Complete Tris Lysis buffer, and prepare samples or dilute cell lysates during this time.

Prepare Complete Tris Lysis Buffer:

a) To 10 mL of Tris Lysis Buffer, add the following:
   - ❑ 200 µL Protease Inhibitor cocktail (50X stock)
   - ❑ 100 µL Phosphatase inhibitor I (100X stock)
   - ❑ 100 µL Phosphatase inhibitor II (100X stock)

b) Keep Complete Tris Lysis Buffer on ice until use.

Prepare samples or positive and negative cell lysates:
(Note: Recommendations for cell lysate handling are provided, however, the suggested concentrations listed below may need to be adjusted depending upon specific samples tested.)

a) Thaw cell lysate samples on ice and dilute immediately before use. Keep on ice during all manipulations and discard all remaining thawed unused material.
b) Dilute cell lysate in Complete Tris Lysis Buffer to a final concentration of 0.4 µg/µL. This will deliver 10 µg/well in 25 µL. A dilution series may also be prepared if desired.

Wash plates four times with Wash Buffer.

STEP 2

Dispense 25 µL/well of samples or diluted lysates.

Incubate with shaking at room temperature for 1 hour. Prepare Detection Antibody Cocktail during this time.

Prepare Detection Antibody:

a) Dilute SULFO-TAG Detection Antibody to a final concentration of 10 nM.
b) Use cold Antibody Dilution Buffer. Sufficient antibody is supplied to prepare 3 mL per plate.

Wash plates four times with Wash Buffer.

STEP 3

Add 25 µL/well of Detection Antibody.

Incubate with shaking at room temperature for 1 hour. Prepare Read Buffer during this time.

Dilute Read Buffer:

a) Approximately 20 mL per plate is required.
b) Dilute 4X Read Buffer T (with surfactant) to 1X with deionized water.

Wash plates four times with Wash Buffer.

Notes:

Plates may also be blocked overnight at 4ºC.

Complete Tris Lysis Buffer should be made each day of experimentation.

The Complete Tris Lysis Buffer should be ice cold before use.

Shaking a 96-well MULTI-ARRAY® or MULTI-SPOT plate accelerates capture at the working electrode.

Diluted Read Buffer may be kept in a tightly sealed container at room temperature for later use.

STEP 4

Add 150 µL of diluted Read Buffer T (with surfactant).

Analyze with SECTOR™ Imager or SECTOR PR™ 400 instrument.

Notes:

Note that bubbles in the Read Buffer will interfere with reliable imaging of the plate if carried into the wells.

Plates can be imaged immediately following the addition of read buffer. Most biological interactions tolerate incubation in Read Buffer however each unique assay should be tested for stability in read buffer before being left to sit for extended periods.