# MSD® 384-Well MULTI-ARRAY®
Phospho-c-Kit (Tyr721) Assay

The following assay protocol has been optimized for quantifying phosphorylated c-kit (Tyr721) in whole cell lysate.

	Storage

MSD Materials

❑ Read Buffer T (with surfactant), 4X	RT
❑ Blocker A	RT
❑ MULTI-SPOT® 384-well 4 Spot Phospho-c-kit plate(s)	2-8ºC
❑ SULFO-TAG™ Anti-Phospho-c-kit (Tyr721) Antibody (50X)	2-8ºC
❑ Tris Wash Buffer (10X)	2-8ºC
❑ Tris Lysis Buffer (1X)	2-8ºC
❑ Phosphatase Inhibitor I (100X)	2-8ºC
❑ Phosphatase Inhibitor II (100X)	2-8ºC
❑ Protease Inhibitor Solution (50X)	≤-10ºC
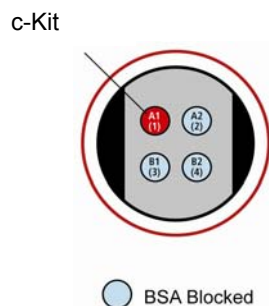
The SECTOR® Imager data file will identify spots according to their well location, not by the coated capture antibody name.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.

Other Materials & Equipment (not supplied)

- ❑ Deionized water for diluting Wash Buffer and Read Buffer
- ❑ One 1 L bottle
- ❑ Two 50 mL tube
- ❑ One 15 mL tube
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker
- ❑ Various microcentrifuge tubes for making serial dilutions of lysates (if desired)
- ❑ Automated plate washer or other efficient multi-channel pipetting equipment for washing 384-well plates
- ❑ Appropriate liquid handling equipment for desired throughput that must accurately dispense 10 μL and 35 μL into a 384-well micro plate

Protocol at a Glance

The following protocol describes the most conservative approach toward achieving highly sensitive results using MSD technology to quantify phosphoproteins. The protocol takes approximately 5 to 5½ 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 2 hours, wash.
3. Add Detection Antibody, incubate 2 hours, wash.
4. Add Read Buffer and analyze plate.

Detailed Instructions

Prepare a stock of 1X Tris Wash Buffer:

a) The stock of 1X Tris Wash Buffer will be used throughout the assay to make other reagents as well as wash plates. Approximately 1 L per plate is required– more if using an automatic plate washer.
b) In a 1 L bottle combine:
- ❑ 100 mL 10X Tris Wash Buffer
- ❑ 900 mL deionized water

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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%)

Prepare Antibody Dilution Buffer:

a) Prepare 8 mL per plate.
b) In a 15 mL tube combine:
    - ❑ 2.67 mL Blocking Solution-A
    - ❑ 5.33 mL 1X Tris Wash Solution

Begin with a MULTI-SPOT 384-well Phospho-c-kit Plate.
No pre-treatment is necessary.

STEP 1

Add 35 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 Solution (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 0.5 μg/μL. This will deliver 5 µg/well in 10 µL. A dilution series may also be prepared if desired.

Wash plates four times with Wash Buffer.

STEP 2   Dispense 10µL/well of samples or diluted lysates.
Incubate with shaking at room temperature for 2 hours. Prepare
Detection Antibody solution during this time.

Shaking a 384-well MSD MULTI-ARRAY or MULTI-SPOT plate accelerates capture at the working electrode.

Prepare Detection Antibody:

In a 15 mL tube combine (per plate):
   a. 7.84 mL cold Antibody Dilution Buffer
   b. 160 µL 50X Anti-Phospho-c-kit (Tyr721) Antibody
      (Final concentration: 1X)

Wash plates four times with Wash Buffer.

STEP 3   Add 10 μL/well of Detection Antibody.
Incubate with shaking at room temperature for 2 hours. Prepare Read
Buffer during this time.

Dilute Read Buffer:

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

In a 50 mL tube, combine (per plate):
   ☐   5 mL 4X Read Buffer T
   ☐   15 mL deionized water

Wash plates four times with Wash Buffer.

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

STEP 4   Add 35 µL of diluted Read Buffer T (with surfactant).
Analyze with SECTOR Imager.

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.

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