

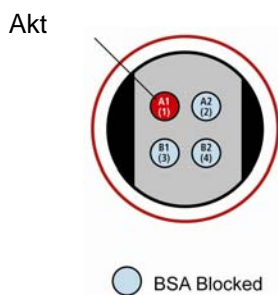
# MSD<sup>®</sup> 96-Well MULTI-ARRAY<sup>®</sup> Phospho-Akt (Thr 308) Assay

The following assay protocol has been optimized for quantifying Phospho-Akt (Thr 308) in whole cell lysate.

Storage

## MSD Materials

<input type="checkbox"/> Read Buffer T (with surfactant), 4X	RT
<input type="checkbox"/> Blocker A	RT
<input type="checkbox"/> MULTI-SPOT <sup>®</sup> 96-well 4-spot Phospho-Akt Plate(s)	2-8°C
<input type="checkbox"/> SULFO-TAG <sup>™</sup> Anti Phospho-Akt (Thr 308) Antibody (50X)	2-8°C
<input type="checkbox"/> Tris Wash Buffer (10X)	2-8°C
<input type="checkbox"/> Tris Lysis Buffer (1X)	2-8°C
<input type="checkbox"/> Phosphatase Inhibitor I (100X)	2-8°C
<input type="checkbox"/> Phosphatase Inhibitor II (100X)	2-8°C
<input type="checkbox"/> Protease Inhibitor Solution (50X)	≤-10°C



The SECTOR<sup>®</sup> 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.



## Notes:

### *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)
- Adhesive plate seals
- Microtiter plate shaker
- Automated plate washer or other efficient multi-channel pipetting equipment for washing 96 well plates
- Appropriate liquid handling equipment for desired throughput that must accurately dispense 25  $\mu$ L and 150  $\mu$ L into a 96-well micro plate.

*Read the entire detailed instructions before beginning work.*

### *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 3 to 3 1/2 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, 1 hour, wash.
2. Add samples or lysate, incubate 1 hour, wash.
3. Add detection antibody, incubate 1 hour, wash.
4. Add Read Buffer and analyze plate.

### *Detailed Instructions*

*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

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



**Notes:**

*Solutions containing Blocker A should be kept at 4°C and discarded after 14 days.*

**Prepare Blocking Solution-A:**

- a) Prepare 25 mL per plate.
- b) In a 50 mL tube combine:
  - 25 mL 1X Tris Wash Buffer
  - 750 mg Blocker A (30 mg/mL or 3%)

**Prepare Antibody Dilution Buffer:**

- a) Prepare 3 mL per plate.
- b) In a 15 mL tube combine (estimated for one plate):
  - 1 mL Blocking Solution-A
  - 2 mL 1X Tris Wash Solution

Begin with a MULTI-SPOT<sup>®</sup> 96-well 4-spot Phospho-Akt Plate. No pre-treatment is necessary.

*Plates may also be blocked overnight at 4°C.*

**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.

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

**Prepare Complete Lysis Buffer:**

- a) Prepare 10 mL Complete Lysis Buffer. 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 Lysis Buffer on ice until use.

*The Complete Lysis Buffer should be ice cold before 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 0.8 µg/µL. This will deliver 20 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.

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

Incubate with shaking at room temperature for 1 hour. Prepare SULFO-TAG Anti Phospho-Akt (Thr 308) Detection Antibody solution during this time.



**Notes:**

*Prepare Detection Antibody Solution:*

- a) Prepare 3.0 mL per plate.
- b) In a 15 mL tube combine:
  - a. 2.94 mL cold Antibody Dilution Buffer
  - b. 60  $\mu$ L 50X Anti Phospho-Akt (Thr 308) Antibody  
(Final concentration: 1X)

Wash plates four times with Wash Buffer.

**STEP 3** Add 25  $\mu$ L/well of Detection Antibody Solution.

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 MSD Read Buffer T (with surfactant) to 1X with deionized water.

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

Wash plates four times with Wash Buffer.

**STEP 5** Prepare SECTOR Imager so that plate can be read immediately following addition of Read Buffer.

Add 150  $\mu$ L/well of diluted Read Buffer T (with surfactant).

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

Analyze with SECTOR Imager, **immediately following addition of Read Buffer.**

*Plates should be imaged immediately following the addition of Read Buffer.*

