MSD® 384-Well MULTI-ARRAY® Total HIF-1α Assay

MSD Materials

	Storage
❑ MULTI-SPOT® 384-well HIF-1α Plate	2-8ºC
❑ SULFO-TAG™ Anti-Total HIF-1α Antibody	2-8ºC
❑ Tris Wash Buffer (10X)	2-8ºC
❑ Tris Lysis Buffer (1X)	2-8ºC
❑ Protease Inhibitor Solution (50X)	≤ -10ºC
❑ Blocker A	RT
❑ Read Buffer T , 4X (with surfactant)	RT

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
- ❑ Various microcentrifuge tubes for making serial dilutions of lysates (if desired)
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker
- ❑ Automated plate washer or other 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

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

Protocol at a Glance

Notes:

The following protocol describes the most conservative approach toward achieving highly sensitive results using MSD technology to quantify HIF-1α. The protocol takes approximately 5 to 51/2 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.

Read the entire detailed instructions before beginning work.

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) 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.

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

b) In a 1 L bottle combine:
   - ❑ 100 mL 10X Tris Wash Buffer
   - ❑ 900 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 8 mL per plate.
b) In a 15 mL tube combine:
   - ❑ 2.67 mL Blocking Solution A
   - ❑ 5.33 mL 1X Tris Wash Buffer

Begin with a MULTI-SPOT Custom 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 Lysis buffer, and prepare samples or dilute cell lysates during this time.

Prepare Complete Lysis Buffer:

a) To 10 mL of Tris Lysis Buffer, add the following:
   - ❑ 200 µL Protease Inhibitor Solution (50X stock)

b) Keep Complete 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 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.

c) Keep diluted cell lysate on ice until use.

Wash plates four times with Tris Wash Buffer.

STEP 2

Dispense 10 µL/well of samples or diluted lysates.

Incubate with shaking at room temperature for 2 hours. Prepare Detection Antibody Cocktail during this time.

Prepare Detection Antibody:

a) Dilute SULFO-TAG Anti-Total HIF-1α Antibody to a final concentration of 10 nM.

b) Use cold Antibody Dilution Buffer. Sufficient antibody is supplied to prepare 8 mL per plate.

Wash plates four times with Tris 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:

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

STEP 4

Add 35 µL/well of diluted Read Buffer T (with surfactant).

Analyze with SECTOR® instrument.