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p-S65-Ub sandwich ELISA

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

Abstract

Here we describe an assay to measure the levels of phosphorylation of Ubiquitin at Serine-65 in vivo by enzyme linked immunosorbent assay (ELISA). This assay was previously described by Watzlawik and colleagues [https://doi.org/10.1080/15548627.2020.1834712].

Materials

- Phospho-Ubiquitin (Ser65) (E2J6T) Rabbit mAb **Cell Signaling Technology Catalog #**62802
- Ubiquitin Monoclonal Antibody (eBioP4D1 (P4D1)), eBioscience™ **Thermo Fisher Scientific Catalog #**14-6078-37
- 200 mM sodium carbonate buffer pH 9.7
- MULTI-ARRAY 96 Plate Pack, SECTOR Plate **MESO SCALE DIAGNOSTICS, LLC. Catalog #**L15XA-3
- **ELISA washing buffer**

	A	B
	Tris, pH 7.4	150 mM
	NaCl	150 mM
	Tween-20	0.1% [v:v]

- **ELISA blocking buffer**

	A	B
	Tris, pH 7.4	150 mM
	NaCl	150 mM
	Tween-20	0.1% [v:v]
	BSA	1% [w:v]

- Anti Mouse Antibody Goat SULFO-TAG Labeled **MESO SCALE DIAGNOSTICS, LLC. Catalog #**R32AC-1
- MSD GOLD Read Buffer A **MESO SCALE DIAGNOSTICS, LLC. Catalog #**R92TG
- microplate mixer (USA Scientific, 8182–2019)

Protocols:

3h

1 Prepare the sodium carbonate buffer, ELISA washing buffer and ELISA blocking buffer.

2 Use rabbit monoclonal p-S65-Ub antibody as capture antibody and used in a final concentration of 1 µg/ml in [M] 200 millimolar (mM) sodium carbonate buffer pH 9.7 and coated Overnight at 4 °C with 30 µL per well in a 96-well MSD plate (MSD, MULTI-ARRAY 96-well Plate; L15XA-3).

3 Wash the next morning MSD plates twice with 0.22-micron filtered ELISA washing buffer using a 1200 µL multi-(12)-channel pipettor with 300 µL per well. Perform the washing by plate inversion and gentle tapping on paper towels (not by pipette aspiration) and with no incubation time between washes.

- ELISA washing buffer

	A	B
	Tris, pH 7.4	50 mM
	NaCl	150 mM
	Tween-20	0.1% [v:v]

4 Block the plates with 0.22-micron filtered ELISA blocking buffer for 01:00:00 at 22 °C without shaking.

1h

- ELISA blocking buffer

	A	B
	Tris, pH 7.4	50 mM
	NaCl	150 mM
	Tween-20	0.1% [v:v]
	BSA	1% [w:v]

5 Run all samples in duplicates and diluted in blocking buffer. Load 30 µg of total protein per well for all mouse tissues in a total volume of 30 µL per well. Adjust the detergent volumes across all samples.

6 Incubate the antigens for 02:00:00 at 22 °C on a microplate mixer (USA Scientific, 8182–2019) at 600 rpm and perform the three washing steps as described before.

2h

7 Subsequently add mouse total Ub antibody (clone P4D1; Thermo Fisher #14-6078-37) as detecting antibody in a final concentration of 5 µg/ml in blocking buffer in 30 µL total volume per well. Incubate detecting antibody for 02:00:00 at 22 °C on a microplate mixer at 600 rpm .

2h

8 After three washing steps, add the SULFO-TAG labeled goat anti-mouse antibody (MSD, R32AC-1) in blocking buffer in a final concentration of 1 µg/ml using 50 µL per well and incubate for 01:00:00 at 22 °C on a microplate mixer at 500 rpm .

1h

9 After another three washing steps, 150 µL MSD GOLD Read Buffer (MSD, R92TG-2) were finally added to each well and the plate being read on a MESO QuickPlex SQ 120 reader.

10 Perform the data analysis in GraphPad prism 9 or similar software.