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Working

Western blot analysis

Version 2

PLOS One

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EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Sumi C, Matsuo Y, Kusunoki M, Shoji T, Uba T, Iwai T, Bono H, Hirota K (2019) Cancerous phenotypes associated with hypoxia-inducible factors are not influenced by the volatile anesthetic isoflurane in renal cell carcinoma. PLoS ONE 14(4): e0215072. doi: 10.1371/journal.pone.0215072

MATERIALS

NAME	CATALOG #	VENDOR
Purified Mouse Anti-Human HIF-1α Clone 54/HIF-1α	610959	BD Biosciences
HIF-2 alpha/EPAS1 Antibody	NB100-122	Novus Biologicals
HIF-1β/ARNT (D28F3) XP® Rabbit mAb	5537	Cell Signaling Technology
Anti α-Tubulin Monoclonal Antibody	017-25031	Fujifilm Wako Pure Chemical
Anti-Mouse IgG HRP-Linked Whole Ab Sheep	NA931	Ge Healthcare
Anti-Rabbit IgG HRP-Linked Whole Ab Donkey	NA934	Ge Healthcare
RIPA buffer	16488-34	Nacalai Tesque
cOmplete™ Protease Inhibitor Cocktail	4693116001	Roche
Blocking One	03953-95	Nacalai Tesque
Chemi-Lumi One Super	02230-14	Nacalai Tesque
DC™ Protein Assay Kit	500-0112	BIO-RAD

MATERIALS TEXT

Antibody dilutions

Primary antibodies

- Anti-HIF-1α 1:1000
- Anti-HIF-2α 1:1000
- Anti-HIF-1β 1:1000
- Anti-α-tubulin 1:2000

Secondary antibodies

- Anti-mouse-IgG 1:10,000
- Anti-rabbit-IgG 1:10,000

Cell lysis

1 Wash the cells with ice-cold PBS and add ice-cold RIPA buffer with cOmplete™ Protease Inhibitor Cocktail.

2 Scrape cells and transfer the suspension to a 1.5 ml microcentrifuge tube.

3 Incubate the lysate on ice for 15 minutes.

4 Centrifuge at 10,000 x *g* for 5 minutes at 4°C and collect the supernatant in a new microcentrifuge tube.

5 Determine protein concentration by using *DC*™ Protein Assay Kit.

Separation of proteins by gel electrophoresis

6 Load 35 µg of total protein on a SDS-PAGE gel.

7 Run the gel for 1 h at 100 V.

Semi-dry membrane transfer

8 Transfer proteins onto a PVDF membrane using Trans-Blot Turbo™ Transfer System.

Western blotting

9 Place the blot in Blocking One and incubate with agitation for 20 minutes.

10 Incubate the blot with appropriate dilutions of primary antibody at 4°C overnight.

11 Place the blot in TBST and wash three times, 5 minutes each.

12 Incubate the blot with the species-matched horseradish peroxidase-conjugated secondary antibody for 1 hour.

13 Place the blot in TBST and wash three times, 5 minutes each.

Chemiluminescent Detection

14 Add Chemi-Lumi One Super to cover the blot and incubate for 1 minute.

15 Detect chemiluminescence using LAS-4000mini image analyzer.