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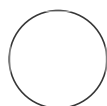
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**Protocol status:** Working  
We use this protocol and it's working

## 🌐 Western blotting of XK and VPS13A V.1

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### ABSTRACT

This protocol describes collection of protein from cultured cells and immunoblotting.

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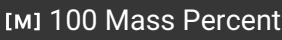

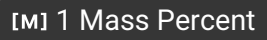
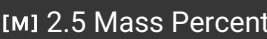


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## Cell culture and treatments












2d 0h 5m

- 1 Culture K562 or COS-7 (ATCC) at  37 °C and 5% CO<sub>2</sub>, using RPMI for K562 or DMEM for COS-7 containing 10% FBS,  1 millimolar (mM) sodium pyruvate,  100 Mass Percent penicillin,  100 Mass Percent streptomycin,  2 millimolar (mM) L-glutamine,  1 Mass Percent non-essential amino acids, (all from Gibco) and  2.5 Mass Percent plasmocin (InvivoGen).
- 2 For K562 cells treated with hemin, supplement the media with hemin (Sigma Aldrich) dissolved in DMSO to a final concentration of  30 micromolar (μM) for  48:00:00 .

## Cell lysis and sample preparation

2d 0h 5m














- 3 Prior to K562 lysis, pellet the cells by centrifuging at  1100 rpm, 4°C, 00:05:00 . Resuspend the pellet in PBS, centrifuging and repeating for a total of 3 times. 5m
- 3.1 Resuspend in PBS and centrifuge  1100 rpm, 4°C, 00:05:00 (1/3). 5m
- 3.2 Resuspend in PBS and centrifuge  1100 rpm, 4°C, 00:05:00 (2/3). 5m
- 3.3 Resuspend in PBS and centrifuge  1100 rpm, 4°C, 00:05:00 (3/3). 5m



- 4 Prior to lysis of confluent COS-7 cells, aspirate media and wash with PBS 3 times.
- 5 Lyse cells with 2% SDS by either resuspending (K562) or adding to culture dish and scraping using a Corning cell-lifter (COS-7). Sonicate lysates using 3x10s pulses with Virsonic 550 (Virtis).
- 6 Centrifuge  13300 rpm, Room temperature, 00:10:00 and collect the post-nuclear supernatant in  new Eppendorf tube.
- 7 Determine protein concentration in sample using Pierce BCA assay (ThermoFisher).
- 8 Prepare samples at desired concentration and add SDS loading buffer to reach a final concentration of  50 millimolar (mM)  06.8 Tris,  2 Mass / % volume SDS,  0.1 Mass / % volume bromophenol blue,  10 % (v/v) glycerol, and  1 % (v/v) beta-mercaptoethanol.
- 9 Boil  95 °C for  00:10:00 . 

## Gel electrophoresis and immunoblotting

1h 15m

- 10 Prepare gel apparatus with 4-12% Tris Glycine gels (Invitrogen) and Tris-Glycine SDS running buffer.
- 11 Load samples into gel and run until dye front reaches bottom (120-150 V).

- 12 Remove gel and set up transfer cassette with nitrocellulose membrane.
- 13 Transfer at 30 V  Overnight at  4 °C in NuPage transfer buffer (Invitrogen)
- 14 Remove nitrocellulose membrane and block membrane with 5% BSA in TBST for  01:00:00 at  Room temperature .
- 15 Add primary antibodies at desired concentration in 5% BSA in TBS-T, incubate  Overnight at  4 °C .
- 16 Wash membrane with TBST. Repeat a total of 3 times.
- 16.1 Wash membrane for  00:05:00 with TBST (1/3). 
- 16.2 Wash membrane for  00:05:00 with TBST (2/3). 
- 16.3 Wash membrane for  00:05:00 with TBST (3/3). 
- 17 Incubate membrane with secondary antibodies conjugated to IRdye 800CW or IRdye 680CW (1:10,000 

Licor) in 5% BSA in TBST for  01:00:00 at  Room temperature .

**18** Wash membrane with TBST. Repeat a total of 3 times.

**18.1** Wash membrane for  00:05:00 with TBST (1/3).

5m

**18.2** Wash membrane for  00:05:00 with TBST (2/3).

5m

**18.3** Wash membrane for  00:05:00 with TBST (3/3).

5m

**19** Image membranes using a Licor Odyssey Infrared Imager.