

Experiment

Test Monoclonal and Polyclonal Figla Ab on lysates

Notebook	SGC-UNC TH FIGLA
Created	Tammy Havener thavener@unc.edu 2023-05-31 15:20:08 GMT
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Content

background

We ordered a Human Ovary Whole Tissue Lysate (NB280-59243) from Novus Biologicals and we have ATCC T47D breast cancer cells to test using the polyclonal and monoclonal Figla antibodies

05/31/23 W blot of lysates

Samples:

T47D cell lysate = 1650ug/ml

Figla P2 (red) protein (3/9/23) = 98.8 mg/ml

Human ovary whole tissue lysate (adult whole normal human) Lot C401165 = 5mg/ml

Run 10ug of each of the samples to probe with antibodies.

1. use 4-20% Tris/Gly wedge well, 15well. With 2xSB and 10xreducing agent.
2. Transfer to PVDF on iBlot, 20v 7min. Cut membrane in half using lane 8

1	T47D lysate, 6ul
2	Figla "+" control, 2ul
3	space
4	Ovary lysate, 4ul
5	Ovary lysate, 2ul
6	space
7	Seeblue 2 MW
8	space
9	T47D lysate, 6ul
10	2Figla "+" control, 2ul
11	space
12	Ovary lysate, 4ul
13	Ovary lysate, 2ul
14	space
15	Seeblue 2 MW

blot 1

- Block in 1%Milkfat/TBST for 1 hr at rt. Add Anti-Figla (PA5-72033) Ab in 5%BSA/TBST, 1:4000dil.
- Goat anti-rabbitHRP in 5%Milkfat/TBST, 1:5000dil, rt, 1 hr
- Run on Bandmate using Ace2 program (1°Ab 8hr, 2°Ab 1hr)

- View with Pierce fempto reagent and view on iBright Image

blot 2

- Block in 1%Milkfat/TBST for 1 hr at rt. Add Anti-Figla (CA151-6A10) Ab in 5%BSA/TBST, 1:1000dil.
- Goat anti-mouseHRP in 5%Milkfat/TBST, 1:2000dil, rt, 1 hr
- Run on Bandmate using Ace2 program (1°Ab 8hr, 2°Ab 1hr)
- View with Pierce fempto reagent and view on iBright Imager

06/01/23 conclusion

Western looks ugly. The Figla protein western on 03/13/23 using 2ul of Figla protein looked better so not sure why this one looks so bad (especially with the polyclonal Ab).

Repeat this and use less of the Figla protein and run it on a lane farther away from the samples. Also, go to 1:5000dil with the polyclonal Ab.

It does appear that the T47D lysate has figla and the ovary lysates also appear to have figla.

06/01/23 W blot repeat

Use 4-20% Tris/Gly wedgewell, 10 well

Transfer to PVDF and cut in half after lane 5

1	Figla "+" control, 1ul
2	space
3	T47D lysate, 10ul
4	Ovary lysate, 8ul
5	Seeblue 2 MW
6	Figla "+" control, 1ul
7	space
8	T47D lysate, 10ul
9	Ovary lysate, 8ul
10	Seeblue 2 MW

blot 1

- Block in 1%Milkfat/TBST for 1 hr at rt. Add Anti-Figla (PA5-72033) Ab in 5%BSA/TBST, 1:5000dil.
- Goat anti-rabbitHRP in 5%Milkfat/TBST, 1:5000dil, rt, 1 hr
- Run on Bandmate using Ace2 program (1°Ab 8hr, 2°Ab 1hr)
- View with Pierce fempto reagent and view on iBright Image

blot 2

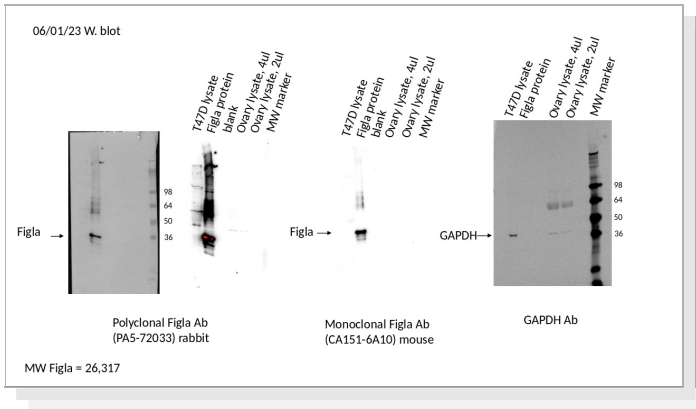
- Block in 1%Milkfat/TBST for 1 hr at rt. Add Anti-Figla (CA151-6A10) Ab in 5%BSA/TBST, 1:1000dil.
- Goat anti-mouseHRP in 5%Milkfat/TBST, 1:2000dil, rt, 1 hr
- Run on Bandmate using Ace2 program (1°Ab 8hr, 2°Ab 1hr)
- View with Pierce fempto reagent and view on iBright Imager

06/01/23 figla Ab western.pptx

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06/02/23 figla Ab western.pptx

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