

(wet) Western Blot Procedures

2015/August/18

Objective: detect the **Top2B** in [different cancer cells](#)

1. Dilute samples into 2 mg/ml

| | | 2 mg/ml final concentration | sample | water |
|----------|---------|-----------------------------|--------|-------------|
| prostate | pc-3 | 23.1 | 4 | 19.1 |
| prostate | rCap | 25.78333333 | 4 | 21.78333333 |
| prostate | LNCap | 29.3 | 4 | 25.3 |
| prostate | Du145 | 26.1 | 4 | 22.1 |
| breast | MCF7 | 33.31666667 | 4 | 29.31666667 |
| breast | MCF12A | 20.8 | 6 | 14.8 |
| breast | T47-D | 26.875 | 6 | 20.875 |
| ovary | OVCAR-3 | 22.175 | 6 | 16.175 |
| ovary | SKOV-3 | 19 | 6 | 13 |

2. Prepare and Run the samples

10 wells, 1 mm, 7.5 % Gel x 2 (with top2B)

| Line | Number | Product name | Company | concentration | Sample amount | Sample Volume | Add 4 x sample buffer | Add DI water |
|------|----------|----------------|----------------|---------------|---------------|---------------|-----------------------|--------------|
| 1 | | Western ladder | | | | 1 ul | | |
| 2 | prostate | PC-3 | From Dr. Irina | 2 mg/ml | 10 ug | 5 ul | 2.5 ul | 2.5 ul |
| 3 | prostate | rCap | From Dr. Irina | 2 mg/ml | 10 ug | 5 ul | 2.5 ul | 2.5 ul |
| 4 | prostate | LNCap | From Dr. Irina | 2 mg/ml | 10 ug | 5 ul | 2.5 ul | 2.5 ul |
| 5 | prostate | Du145 | From Dr. Irina | 2 mg/ml | 10 ug | 5 ul | 2.5 ul | 2.5 ul |
| 6 | breast | MCF7 | From Dr. Irina | 2 mg/ml | 10 ug | 5 ul | 2.5 ul | 2.5 ul |
| 7 | breast | MCF12A | From Dr. Irina | 2 mg/ml | 10 ug | 5 ul | 2.5 ul | 2.5 ul |
| 8 | breast | T47-D | From Dr. Irina | 2 mg/ml | 10 ug | 5 ul | 2.5 ul | 2.5 ul |
| 9 | ovary | OVCAR-3 | From Dr. Irina | 2 mg/ml | 10 ug | 5 ul | 2.5 ul | 2.5 ul |
| 10 | ovary | SKOV-3 | From Dr. Irina | 2 mg/ml | 10 ug | 5 ul | 2.5 ul | 2.5 ul |

3. Transfer the protein to the membrane

Transfer buffer: Tris base 48 mM, Glycine 39 mM, 0.04% SDS, 20% methanol, 1 h, 100 V

4. Antibody information

| | | | | | | | | | | |
|------------|-----|------|-------------------|------|------------|--------|-----------|--------|---------|--------|
| Anti-top2B | New | Good | Novusbio, ab52934 | 4 °C | polyclonal | Rabbit | 0.2 mg/ml | 183 KD | 1: 1000 | 100 ul |
|------------|-----|------|-------------------|------|------------|--------|-----------|--------|---------|--------|

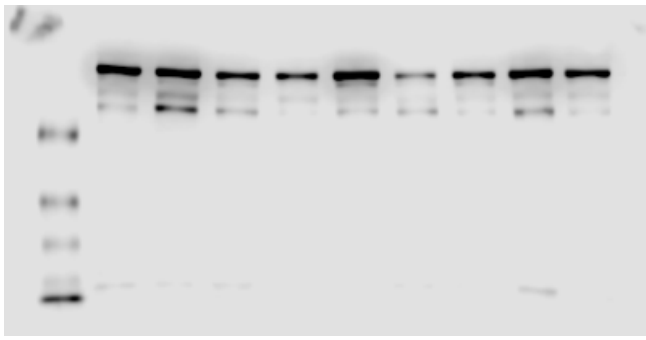
5. Blocking and Adding antibodies ([Keep TBST cold 4°C](#))

14 h, incubation with primary antibody

6. Stain membrane

Should let the membrane dry a little bit, because that it easy to stain on the protein lanes

Results



Novusbio, top2B