

## HER2 Immunoprecipitation

### INTRODUCTION

When a protein is expressed at low levels and is difficult to detect with western blot analysis, immunoprecipitation (protein pull down) may be the method of choice. An immunoprecipitating reagent has to be specific in order to avoid precipitation of unwanted protein. Furthermore, sufficient affinity is required to pull down the protein and to withstand stringent washing steps. The Anti-HER2 Affibody<sup>®</sup> molecule is a specific affinity ligand and has been proven well suited for pull down experiments of HER2 proteins.

### RESULTS IMMUNOPRECIPITATION

Cell extracts prepared from SK-BR3, HEP-G2 and SH-SY5Y cells were incubated with agarose immobilized Anti-HER2 Affibody<sup>®</sup> molecule for 2 hours. After incubation, the unbound proteins were washed away and the bound protein was eluted with SDS-PAGE separation and blotted onto a PVDF filter. The filter was stained with an antibody against the intracellular domain of the HER2 receptor. The antibody binds to both the full-length protein (150 kDa) and to a splice variant (approximately 100 kDa) of the receptor that has been reported to be produced by several cell lines.

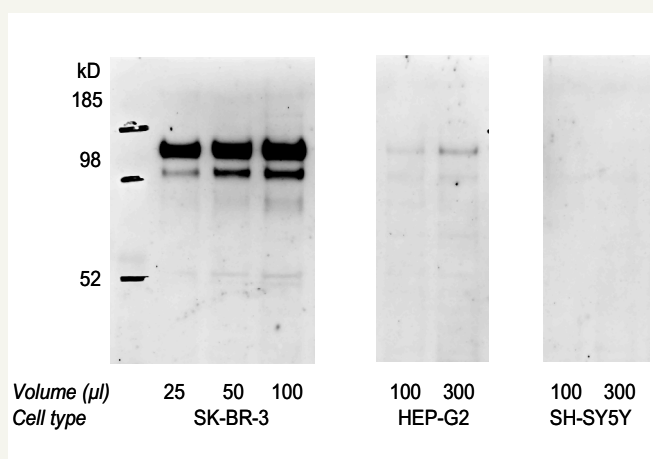


Fig. 1. The agarose immobilized Anti-HER2 Affibody<sup>®</sup> molecule precipitates HER2-protein from cell extracts derived from HER2 positive cell lines SK-BR-3 and HEP-G2, but not from the HER2 low expressing SH-SY5Y cell line.

Figure 1a shows that the HER2 proteins were precipitated from different volumes of SK-BR3 cell extract using the agarose immobilized Anti-HER2 Affibody<sup>®</sup> molecule. An

intense HER2 band was obtained on the Western blot from as little as 25 μl extract. With increased volume of extract, the HER2 band became even more intense. HER2 proteins were also precipitated from HEP-G2 cell extract, shown in figure 1b. A relatively weak band was obtained when precipitating from 300 μl extract indicating that HEP-G2 expresses HER2 to a lower level than SK-BR3. No bands were visible after precipitation with SH-SY5Y cell extracts, shown in figure 1c, suggesting that this cell line has a very low level of HER2 expression.

In summary, the Anti-HER2 Affibody<sup>®</sup> molecule efficiently precipitates HER2 from a complex protein mix. When performing immunoprecipitation experiments with antibodies, there is often a problem with cross reaction between the enzyme conjugated second step reagent and the precipitating antibody but this type of cross reaction is elegantly avoided using an Affibody<sup>®</sup> molecule as the precipitating reagent.

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### MATERIALS AND BUFFERS REQUIRED

**Precipitating agent:** Anti-HER2 Affibody® IP kit (Agarose immobilized Anti-HER2 Affibody® molecule and cysteine blocked agarose (Affibody cat no 10.0817.04.9002))

**PBS:** 2.68 mM KCl, 1.47 mM KH<sub>2</sub>PO<sub>4</sub>, 137 mM NaCl, 8.1 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 7.4

**Lysis buffer (RIPA):** 50 mM Tris-HCl pH 7.4, 1% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl, 1 mM EDTA

**Protease inhibitors:** Complete Mini, EDTA free (Roche Diagnostics)

**Tubes:** 1.5 ml conical tube (Eppendorf), 50 ml tubes (Falcon)

### PREPARATION OF CELL EXTRACT

**Note:** Work on ice or in a cold room.

1. Wash the cells with ice cold PBS.
2. Count the cells.
3. Wash the cells with ice cold PBS and remove excess PBS from the cell pellet.
4. Lyse cells with RIPA buffer containing protease inhibitors. Prepare cell extract with at least 10 x 10<sup>6</sup> cells per ml lysis buffer. If low HER2 protein expression is anticipated, use a higher concentration extract by increasing the number of cells per ml.
5. Pipette the cell pellet and transfer the lysate to an Eppendorf tube.
6. Incubate the cell extract at +4°C by gently rocking or by end over end rotation for 30 minutes.
7. Spin the lysate for 15 minutes at 10 000\*g at +4°C. Use a cooled centrifuge.
8. Remove the supernatant (cell extract) carefully to a new Eppendorf tube.
9. The cell extract is now ready for pre-clearing or alternatively, storage at -80°C.

### PRE-CLEARING OF CELL EXTRACT

**Note:** To reduce unspecific binding of proteins to the agarose matrix, a pre-clearing step may be needed. Work on ice or in a cold room.

1. Wash the cysteine blocked agarose twice with RIPA buffer.
2. Add 1 ml of cell extract per 100 µl packed cysteine blocked agarose.

3. Incubate with end over end rotation for 1 hour at +4°C.
4. Spin for 30 seconds at a maximum of 5000\*g. Use a cooled centrifuge.
5. Collect the supernatant (the pre-cleared extract), and transfer it to a new Eppendorf tube. Discard the cysteine blocked agarose. Measure the total protein content with a BCA test or similar.
6. The pre-cleared cell extract is now ready for precipitation experiments or store at -80°C.

### PULL DOWN

1. Mix the pre-cleared cell extract with 15-30 µl of packed agarose-immobilized Anti-HER2 Affibody® molecule.  
**Note:** The volume of cell extract and the amount of matrix should be determined by the user. The combination of 30 µl packed matrix with an end volume of 300 µl is recommended. A smaller volume may make mixing difficult.
2. Incubate the mix for 1-2 hours at +4°C by end over end rotation.
3. Spin the mix at a maximum of 5000\*g for 30 seconds. Use a cooled centrifuge.
4. Discard the supernatant.
5. Wash by adding 1 ml RIPA buffer containing protease inhibitors and spin the mix at a maximum of 5000\*g for 30 seconds. Use a cooled centrifuge.
6. Discard the supernatant. Repeat the washing steps 4 times.
7. Resuspend the mix in 30 µl of sample buffer. Boil for 10 minutes.
8. Analyze the sample immediately by Western blot, or store at -20°C.

### WESTERN BLOT

For SDS-PAGE separation and transfer, the Novex NuPAGE system (4-12% gels) and the Novex transfer system were used. The proteins were blotted onto Immobilon PVDF membranes (Millipore) and the membranes were blocked with PBST + 0.5% casein. Western Blot staining of HER2 was done with rabbit anti-cErbB2 antibody (Neomarker Lab Vision Corp.), followed by HRP conjugated anti-rabbit IgG (Dako). Super Signal West-Dura (Pierce) was used as substrate for chemiluminescence. Images of the blot was taken with a Chemilmager (Alpha Innotech Corp.).

### LIMITATIONS

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