Product datasheet

Anti-ErbB2 Affibody® Molecule ab31889

Overview

**Product name**
Anti-ErbB2 Affibody® Molecule

**Specificity**
This product binds to the extracellular domain of human ErbB2. A head-to-tail dimer through its peptide bond.

**Tested applications**

- **Suitable for:** IHC-Fr, ICC/IF, Flow Cyt
- **Unsuitable for:** IHC-P

**Species reactivity**

- **Reacts with:** Human

**Immunogen**

This product is a recombinant protein produced in E.coli.

**General notes**

**What are Affibody Molecules?**

Affibody® affinity ligands are unique research reagents, produced using innovative protein-engineering technologies. They are small, simple proteins composed of a three-helix bundle based on the scaffold of one of the IgG-binding domains of Protein A. Protein A is a surface protein from the bacterium Staphylococcus aureus. This scaffold has excellent features as an affinity ligand and can be designed to bind with high affinity to any given target protein. The domain consists of 58 amino acids, 13 of which are randomized to generate Affibody® libraries with a large number of ligand variants. Thus, the libraries consist of a multitude of protein ligands with an identical backbone and variable surface-binding properties. In function, Affibody® Molecules mimic monoclonal antibodies. Compared to antibodies, the most striking dissimilarity of Affibody® Molecules is the small size. Affibody® Molecules have a molecular weight of 6kDa, compared to the molecular weight of antibodies, which is 150kDa. In spite of it’s small size, the binding site of Affibody® Molecules is similar to that of an antibody. The advantages of Affibody® Molecules over antibodies are: -their small size -the simple structure of the molecules -its robust physical properties; able to withstand a broad range of analytical conditions, including extreme pH and elevated temperature -its ability to fold correctly intracellularly -the fast and cost effective production in bacteria -the potential to couple Affibody® Molecules in multimeric constructs Affibody® Molecules have highly competitive properties for applications within affinity purification, sample preparation, protein detection and in vitro diagnostics.

ab50345 is a secondary antibody suitable for use in the process of detecting this Affibody® Molecule. This Anti-ErbB2 Affibody® Molecule is modified with a unique C-terminal cysteine for directed single-point chemical modification, facilitating labelling with fluorescent dyes, biotin or coupling to matrices. However, tail-to-tail dimers are spontaneously generated via a disulphide bridge between the C-terminal cysteines. Prior to coupling via the C-terminal the Affibody®
Molecule needs to be reduced to expose the reactive cysteine residue. Recommended reducing condition is 20mM DTT at a pH above 7.5 and incubation at room temperature for 2 hours. Remove excess DTT by passage through a desalting column, not by dialysis.

**THIS AFFIBODY® MOLECULE REQUIRES CONJUGATION TO A SUITABLE LABEL BEFORE USE. PLEASE REFER TO THE "PROTOCOLS" LINK BELOW.**

### Properties

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| Storage buffer | pH: 7.40  
Constituents: PBS, 0.079% Ammonium bicarbonate |
| Purification notes | The purity of this product is >98% as determined by SDS-PAGE (Coomassie blue staining) and RP-PLC analysis. |

### Function

Protein tyrosine kinase that is part of several cell surface receptor complexes, but that apparently needs a coreceptor for ligand binding. Essential component of a neuregulin-receptor complex, although neuregulins do not interact with it alone. GP30 is a potential ligand for this receptor. Regulates outgrowth and stabilization of peripheral microtubules (MTs). Upon ERBB2 activation, the MEMO1-RHOA-DIAPH1 signaling pathway elicits the phosphorylation and thus the inhibition of GSK3B at cell membrane. This prevents the phosphorylation of APC and CLASP2, allowing its association with the cell membrane. In turn, membrane-bound APC allows the localization of MACF1 to the cell membrane, which is required for microtubule capture and stabilization. In the nucleus is involved in transcriptional regulation. Associates with the 5'-TCAAATTC-3' sequence in the PTGS2/COX-2 promoter and activates its transcription. Implicated in transcriptional activation of CDKN1A; the function involves STAT3 and SRC. Involved in the transcription of rRNA genes by RNA Pol I and enhances protein synthesis and cell growth.

### Tissue specificity

Expressed in a variety of tumor tissues including primary breast tumors and tumors from small bowel, esophagus, kidney and mouth.

### Involvement in disease

Hereditary diffuse gastric cancer  
Glioma  
Ovarian cancer  
Lung cancer  
Gastric cancer  
Chromosomal aberrations involving ERBB2 may be a cause gastric cancer. Deletions within 17q12 region producing fusion transcripts with CDK12, leading to CDK12-ERBB2 fusion leading to truncated CDK12 protein not in-frame with ERBB2.

### Sequence similarities

Belongs to the protein kinase superfamily. Tyr protein kinase family. EGF receptor subfamily. Contains 1 protein kinase domain.

### Post-translational modifications


### Cellular localization

Applications

Our Abpromise guarantee covers the use of ab31889 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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**Application notes** Is unsuitable for IHC-P.

Images

- **Immunocytochemistry/Immunofluorescence - Anti-ErbB2 Affibody® Molecule (ab31889)**
  - Fluorescence staining of cells
    - The human mammary gland cell line SK-BR3 expresses high levels of ErbB2 and this cell line was used to demonstrate Affibody® fluorescence staining and to compare three different staining reagents for cells. Fluorescein conjugated (a), biotin conjugated (b) and Oregon Green® (c) labeled Affibody® molecule were used as reagents in this experiment. The SK-BR3 cells were stained for 30 minutes at a concentration of 1-5 μg Affibody® molecule/ml. Figure 1 a-c shows bright membrane staining with all three reagents. As both the fluorescein conjugated and Oregon Green® labeled Affibody® molecule function as one step reagents, the staining procedure was completed in only 30 minutes. Nuclei were counter stained with DAPI (blue fluorescence).

- **Immunohistochemistry (Frozen sections) - Anti-ErbB2 Affibody® Molecule (ab31889)**
  - Fluorescence staining of frozen tissue sections
    - Frozen tissue sections were obtained from snap frozen xenograft SK-OV-3 tumors. The sections were stained with Oregon Green® conjugated Anti-ErbB2 Affibody® molecule for 30 minutes at a concentration of 2 μg Affibody® molecule/ml. The resulting microscope image shows brightly stained SK-OV-3 cells inside the tumor whereas the connective tissue surrounding and traversing the tumor cells remained negative.
Immunohistochemistry (Frozen sections) - Anti-ErbB2 Affibody® Molecule (ab31889)

Immunohistochemical staining of frozen tissue sections
Xenograft tumors of the human ovarian adenocarcinoma, SK-OV-3 was soaked in formaldehyde and then snap-frozen in liquid nitrogen and used for immunohistochemical staining with the HRP-conjugated Anti-ErbB2 Affibody® molecule. Frozen tissue sections were stained with HRP-conjugated Anti-ErbB2 Affibody® molecule for 45 minutes at room temperature. The staining was developed with DAB substrate and the tissue sections were counter stained with Mayer’s Haematoxylin. The resulting microscope image shows strong brown membrane staining of tumor cells in the xenograft whereas the mouse connective tissue that surrounds and traverses the tumor remains negative. Thus, the HRP-conjugated Anti-ErbB2 Affibody® molecule is a rapid reagent for ErbB2 specific immunohistochemical staining of frozen tissue sections.

Flow Cytometry - Anti-ErbB2 Affibody® Molecule (ab31889)

Flow cytometry analysis of ErbB2 expression
The Oregon Green®-conjugated Anti-ErbB2 Affibody® molecule was used as a one step detection reagent for analysis of ErbB2 expression using flow cytometry. Cells from the ErbB2 positive human ovarian cancer cell line SK-OV-3 and the ErbB2-negative human neuroblastoma cell line SH-SYSY were stained with the Oregon Green®-conjugated Anti-ErbB2 Affibody® molecule. As shown in figure a, staining with Oregon Green®-conjugated Anti-ErbB2 Affibody® molecule resulted in increased fluorescence intensity and the whole cell population of SK-OV-3 cells was shifted to the right (red line) compared to the control (black line). On the contrary, Anti-ErbB2 Affibody® molecule staining of the ErbB2 negative cell line SH-SYSY did not cause a shift in fluorescence intensity, as shown in figure b.

Please note: All products are “FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES”

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