**Polyclonal Rabbit Anti-Human c-erbB-2 Oncoprotein**
**Code No. A 0485**
**Lot 041. Edition 22.05.01**

**Intended use**
For in vitro diagnostic use.

DAKO Polyclonal Rabbit Anti-Human c-erbB-2 Oncoprotein is intended for use in immunocytochemistry. The antibody labels normal epithelial cells, which, however, generally express c-erbB-2 protein at a very low level. It is a useful tool for the identification of overexpression of c-erbB-2 oncoprotein in a variety of epithelial neoplasms, for example subsets of breast carcinomas, pulmonary adenocarcinomas, colorectal adenocarcinomas, pulmonary squamous and gastric adenocarcinomas (1), transitional cell carcinomas of the urinary bladder (2), and endometrial adenocarcinomas (3). Differential identification is aided by the results from a panel of antibodies. Interpretation must be made within the context of the patient’s clinical history and other diagnostic tests by a qualified pathologist.

**Synonyms for antigen**
HER2 (human epidermal growth factor receptor 2) (4), HER2/neu (5), ErbB2 (4, 5) and p185HER2 (6).

**Introduction**
c-erbB-2 oncoprotein is a 185 kDa transmembrane tyrosine kinase belonging to the epidermal growth factor receptor (EGFR) family. This family comprises four homologous receptors ErbB-1 (EGFR, HER1), ErbB-2 (HER2/neu), ErbB-3 (HER3), and ErbB-4 (HER4) (4). The c-erbB-2 proto-oncogene is located on chromosome 17 at q21 (7). Activation of c-erbB-2 oncoprotein, either by homo- or heterodimerization, triggers intracellular signalling events, which are crucial for cell growth, differentiation and survival. Mechanisms promoting receptor dimerizations include ligand binding and high receptor density (overexpression). An overexpression of c-erbB-2 oncoprotein is often a result of gene amplification (4).

c-erbB-2 oncoprotein is frequently overexpressed in human carcinomas, thus 25-30% of human breast carcinomas overexpress this receptor (6, 8), whereas overexpression has not been found in benign breast disease (4).

In the last two decades, monoclonal antibodies which block activation of c-erbB-2 oncoprotein have been developed and clinical activity of a humanized monoclonal antibody, Herceptin™, has been documented (6).

**Reagent provided**
Affinity-isolated rabbit antibody purified by using immobilized c-erbB-2 oncoprotein peptide and provided in liquid form. In 0.05 mol/L Tris/HCl, 0.1 mol/L NaCl, 15 mmol/L NaN₃, pH 7.2. Package size is 0.2 mL.

Antibody concentration: 0.5 g/L.

Note: As of lot 041 the antibody concentration has been reduced by 50%.

**Immunogen**
Synthetic human c-erbB-2 oncoprotein peptide from the intracytoplasmic part of the c-erbB-2 oncoprotein. The peptide was coupled to keyhole limpet hemocyanin (KLH).

**Specificity**
The antibody labels an intracellular domain of c-erbB2 oncoprotein.

In immunocytochemistry, formalin-fixed, paraffin-embedded breast carcinoma cell lines expressing c-erbB-2 oncoprotein at levels 1+ (MDA-175) and 3+ (SK-BR-3), display the expected labelling intensity of the cell membranes with the antibody. The breast carcinoma cell line, MDA-230, which expresses c-erbB-2 oncoprotein at very low level is consistently negative.

**Precautions**
1. For in vitro diagnostic use.
2. The NaN₃ used as a preservative is toxic if ingested. NaN₃ may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing.

**Storage**
Store at 2-8 °C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the user must verify the conditions. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact DAKO Technical Services.

**Specimen preparation**
**Paraffin sections:** The antibody can be used on paraffin-embedded tissue sections fixed in formalin. Heat-induced epitope retrieval in 10 mmol/L citrate buffer, pH 6.0, or in DAKO Target Retrieval Solution, code No. S 1700, is recommended. The tissue sections should not dry out during the treatment or during the following immunocytochemical staining procedure.

(2)
Frozen sections and cell smears: Not tested.

**Staining procedure**

**Dilution:** DAKO Polyclonal Rabbit Anti-Human c-erbB-2 Oncoprotein, code No. A 0485, may be used at a dilution range of 1:250-1:350 when applied on formalin-fixed, paraffin-embedded sections of human mammary carcinoma overexpressing the c-erbB-2 oncoprotein, and using 15 minutes heat-induced epitope retrieval in 10 mmol/L citrate buffer, pH 6.0, and 30 minutes incubation at room temperature with the primary antibody. Optimal conditions may vary depending on specimen and preparation method, and should be determined by each individual laboratory. As negative control, DAKO Rabbit Immunoglobulin Fraction (Solid-Phase Absorbed) code No. X 0936, diluted to a protein concentration identical to the antibody concentration of the primary antibody, is recommended.

**Visualization:** DAKO LSAB®+/HRP kits, code Nos. K 0679 and K 0690, and DAKO EnVision™+/HRP kits, code Nos. K 4008 and K 4010, are recommended. Follow the procedure enclosed with the selected visualization kit.

**Automation:** The antibody is well-suited for immunocytochemical staining using automated platforms, such as the DAKO Autostainer.

**Product-specific limitations**

Occasional cytoplasmic staining may be observed. It should be disregarded and only staining of the cell membrane should be considered specific for c-erbB-2 oncoprotein.

**Performance characteristics**

Cells labelled specifically by the antibody display a staining confined to the cell membrane.

**Normal tissues:** c-erbB-2 oncoprotein is a normal tissue component and the antibody may display a weak labelling of normal epithelial cells. Squamous epithelium in the esophagus and tonsil may in some cases show moderate staining. Prostatic gland tissue has also been found moderately positive. A long range of other normal tissues, such as adrenal gland, bone marrow, brain, heart, liver, lung, skeletal muscle, skin, spleen, thymus and thyroid gland, is negative.

**Abnormal tissues:** In one study (1), 13/59 (22%) of breast carcinomas, 8/29 (28%) pulmonary adenocarcinomas, 10/58 (17%) colorectal adenocarcinomas, and 6/56 (11%) pulmonary squamous and 7/62 (11%) gastric adenocarcinomas showed c-erbB-2 oncoprotein overexpression (complete membrane staining – weak to strong – in more than 10% of the tumour cells) when tested with the antibody. In another study (2) 101/177 (57%) of transitional cell carcinomas of the urinary bladder showed c-erbB-2 oncoprotein overexpression. Of endometrial adenocarcinomas of endometrioid type 15/112 (13%) overexpressed c-erbB-2 oncoprotein (3). No overexpression was found in 30 kidney adenocarcinomas, 12 hepatocellular carcinomas, and 17 malignant melanomas (1).

**References**


A 0485/HEW/22.05.01