CE IVD

BioSite Histo HER2 easy Kit IHC

REF No BCB-20054 ca. 60 tests

Instructions for use

Intended use

HER2easy Kit IHC is designed for semi-quantitative immunohistochemical detection of HER2 protein expression in formalin-fixed, paraffin-embedded tissue sections. HER2easy Kit IHC is intended for in vitro diagnostic use.

Summary and Description

HER-2/neu protein (*Human Epithelial Growth Factor Receptor* 2) is a 185 kDa transmembrane glycoprotein with tyrosine kinase activity. It belongs to the protein family of epidermal growth factor receptors and has a high homology to the epidermal growth factor receptor 1 (EGFR1 or HER1). Synonyms for HER2 protein are CD380, EGFR2, or p185. The gene is also described as *HER2*, *ERBB2* or *NEU*. The HER2 protein is expressed in a variety of epithelial cells (1-6).

A certain fraction of breast cancer patients show overexpression of the HER2 gene. This overexpression is considered to be a reason for the malignant transformation and tumour progression. Proof of HER2 overexpression in breast carcinoma is regarded to be necessary for a therapy with Trastuzumab (Herceptin™) (7-9).

Principle of the method

Paraffin-embedded tissue sections are first dewaxed and rehydrated. Endogenous peroxidase activity within the tissue may cause non-specific staining. This enzyme activity can be blocked by incubation with 3% H_2O_2-solution (Peroxide Block).

The next step is incubation with the primary antibody specific to the HER2 protein.

After washing, the HRP Polymer is applied and incubated. The HRP Polymer in this kit consists of secondary antibodies and horse radish peroxidase (HRP) molecules covalently bound to a polymer backbone. Any excess of unbound HRP Polymer is thoroughly washed away after incubation.

The addition of the chromogenic substrate starts the enzymatic reaction of the peroxidase which leads to colour precipitation where the primary antibody is bound. The chromogen used determines the colour. The chromogen DAB which is included in this kit forms a dark brown precipitate.

The colour can be observed by light microscopy after counterstaining and blueing.

Reagent provided and storage conditions

Reagent	Format	Volume	Storage
HIER citrate Buffer pH 6.0 (10X)	10X Concenrate	2 x 100 ml	2-8 °C
Wash Buffer (20X)	20X Concentrate	500 ml	18-25 °C
Perioxide Block	Ready to-use	8 ml	2-8 °C
Mouse monocolonal antibody to HER2/neu (c-erbB2) clone CB11	Ready to-use	6 ml	2-8 °C
HRP Polymer anti-Mouse/Rabbit/Rat	Ready to-use	6 ml	2-8 °C
DAB Chromogen	Concentrate	3 ml	2-8 °C
DAB Chromogen	Ready to-use	8 x 5 ml	2-8 °C

Materials required but not provided
Adhesive slides and cover slips
Positive und negative control tissue
Xylene or xylene substitutes
Ethanol, distilled H2O
Device for heat pre-treatment (pressure cooker, steamer)
Pipettes, Coplin jars, measuring glasses, incubation chamber (“humidity chamber”)
PAP Pen (Cat. No. LP0001)
Negative control reagent
Counterstain solution
Mounting medium

Storage and handling
The reagents should be stored according to the recommendations on the product labels without further dilution.
Please store the reagents in a dark place and do not freeze them. Under these conditions the solutions are stable up to the indicated expiry date. They must not be used after the expiry date.
All reagents should be stored at 2-8°C. Only the Wa sh Buffer (*Wash Buffer 20X*) has to be kept at room temperature (18-25°C). If the Wash Buffer 20X concentrate is st ored in a fridge salts will precipitate. They will be dissolved when the
Wash Buffer has reached room temperature. Thereafter the Wash Buffer can be used without restrictions. The diluted working strength solution is stable for at least 1 week if stored at 2-8°C.
If reagents are stored under any conditions other than those specified, they must be validated by the user.
Since there are no obvious indicators for instability of the reagents, positive and negative controls should always 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 kit reagents is suspected, contact Biosite Histo Systems’ technical support or your local distributor.

Precautions and other recommendations
1. Use through qualified personnel only. Material safety data sheets (MSDS) are available upon request for qualified personnel.
2. For in vitro diagnostic use.
3. Wear personal protective equipment to avoid contact of reagents and specimens with eye, skin and mucous membranes. If reagents or specimens come in contact with sensitive area, wash with large amounts of water.
4. The reagents of this kit contain preservatives for stabilisation. ProClin300 and sodium azide (NaN_3) are used for stabilisation. Reaction of sodium azide with lead or copper in drainage pipes can result in the formation of highly explosive metallic azides. Sodium azide should be discarded in a large volume of running water to avoid formation of deposits. Material safety data sheets (MSDS) for the pure substances are available upon request.
5. *Peroxide Block* contains 3% hydrogen peroxide (H_2O_2). The material safety data sheet for the pure substance is available upon request.
6. *Mouse monoclonal antibody HER2/neu* and *HRP Polymer anti-Mouse/Rabbit/Rat* contain material of animal origin. Like all other products obtained from biological materials they have to be used correctly.
7. *DAB Chromogen* contains 3,3’-Diaminobenzidin-tetrahydrochlorid. It can cause chemical burns and may cause cancer. The material safety data sheet is available upon request.
8. Microbial contamination of the reagents must be avoided, since otherwise non-specific staining may occur.
9. Dilutions, incubation conditions and technical methods different from those recommended can lead to incorrect results.
10. Please store the reagents in a dark place and do not expose them to strong light. The staining reaction itself can be influenced when carried out in strong light for example in direct sunlight.
11. All kit components are formulated specifically for use with this test and should not be substituted by other reagents without a new validation. Only the Wash Buffer (*Wash Buffer 20 X*) and the Citrate Buffer (*HIER Citrate*
12. *Buffer*) can be replaced by the corresponding single reagents BCB-20021/22 and BCB-20015/16.

Staining procedure
Reagent preparation
A. Reagent preparation

1. Preparation of the pre-treatment solution: Dilute *HIER Citrate Buffer pH 6.0 (10 X)* 1:10 with deionised or distilled water and mix thoroughly.
2. Preparation of the wash buffer: Dilute *Wash Buffer (20 X)* 1:20 with deionised or distilled water and mix thoroughly.
3. Preparation of chromogenic substrate working solution: Add 5 drops (ca. 200 µl) of DAB Chromogen (*DAB concentrate*) to one bottle of DAB Substrate Buffer (5 ml *DAB Substrate Buffer*) and mix thoroughly. This working solution is stable for at least six hours but must be protected from strong light.

B. Pre-treatment of FFPE tissue slides (HIER, Heat Induced Epitop Retrieval)

Paraffin-embedded tissue sections should be placed on adhesive slides. Deparaffinise and rehydrate sections and pretreat them according to procedure I (pressure cooker) or II (steamer) for epitope retrieval.

Procedure 1: Pressure cooker

1. Fill pressure cooker with demineralised or distilled water. You need ca. 1.5 litre water for most pressure cookers.
2. Fill Coplin jar(s) with prepared working strength solution of Citrate Buffer. Make sure there is enough solution tocover the tissue sections on the slides completely. Place Coplin jar(s) with Citrate Buffer in the pressure cooker andplace the lid loosely on the cooker.
3. Heat pressure cooker with lid in place but not closed on a heating plate until the pre-treatment solution reaches 95°C.
4. Place slides with tissue sections into the preheated solution. Cover the Coplin jars loosely with lids. Tissue sections must be completely covered with Citrate Buffer solution.
5 Close the lid of the pressure cooker and heat until maximum pressure is reached. Usually two rings of the pressuregauge are visible.
6 Leave the pressure cooker for additional 10 minutes on the heating plate. Hence, the complete cooking time is 10 minutes.
7 Switch off heating plate. Place the pressure cooker under running tap water until it is cooled down and the excess pressure is relieved.
8 Open the now non-pressurised cooker and let cold tap water run in until all water inside as well as the pre-treatment solution in the jars is replaced by tap water.
9 Transfer tissue slides into Wash Buffer and start with immunohistochemical staining.

10 **Procedure II: Steamer**

1. Fill steamer up to the mark “maximum“ with tap water and heat with lid closed until the water is boiling (ca. 10 minutes).
2. Fill Coplin jar(s) with prepared working strength solution of Citrate Buffer. Please make sure there is enough solution to cover the tissue sections on the slides completely. Place Coplin jar(s) with Citrate Buffer in the steamer and place the lid(s) loosely on Coplin jar(s).
3. Close steamer and heat for 30 minutes to preheat the pre-treatment solution inside the Coplin jar(s).
4. Check the temperature in the pre-treatment solution. It should be >/= 95°C. Place slides with tissu e sections into the pre-heated solution. Cover the Coplin jars loosely with lids. Tissue sections must be completely covered with Citrate Buffer solution.
5. Close steamer and heat again for 30 minutes.
6. Switch off steamer, remove Coplin jar(s) and let them cool down for 20 minutes on the laboratory bench.
7. Let tap water run slowly into the jar(s) until all pre-treatment solution is replaced by tap water.
8. Transfer tissue slides into Wash Buffer and start with immunohistochemical staining.

B. Imminohistochemical staining procedure

- The tissue sections have to be completely deparaffinised and pre-treated for epitope retrieval (HIER).
- Reagents should be at room temperature when used (18 – 25°C).
- All incubation steps should be carried out at room temperature. Tissue sections have to be completely covered with the different reagents in order to avoid drying out.

1. *Optional*: Encircle tissue sections with Pap Pen (i.e. LP0001)
2. *Peroxide Block* (3% H_2O_2 solution) **10 min.**
3. Wash with Wash Buffer **1 x 2 min.**
4. HER2 antibody (*Mouse monoclonal antibody HER2/neu*)
or negative control reagent **60 min.**
5. Wash with Wash Buffer **3 x 2 min.**
6. *HRP Polymer anti-Mouse/Rabbit/Rat* **30 min.**
7. Wash with Wash Buffer **3 x 2 min.**
8. DAB **10 min.**
9. Stop the reaction with distilled water
10. Counterstaining and blueing
11. Mounting: aqueous or permanent mounting after dehydration in a graded series of ethanol and clear in xylene

Explanations of the symbols on the products:

REF	Catalog Number Bestellnummer Reference du catalogue	LOT	Batch Code Chargenbezeichnung Code du lot	Manufacturer Nordic BioSite AB Propellervägen 4A S-183 62 Täby Sweden Tel: +46 (0)8 5444 33 40 Fax: +46 (0)8 756 94 90 info@biosite.se www.biosite.se
	Use By Verwendbar bis Utiliser jusque	IVD	In Vitro Diagnostic Medical Device In vitro Diagnostikum Dispositif médical de diagnostic in vitro	
	Consult Instructions for use Gebrauchsanweisung beachten Consulter les instructions d'utilisation		Temperature Limitation Lagerungstemperatur Limites de température	

Quality control
Variations in fixation, embedding and other processing steps of the tissues to be analysed can cause inconsistent staining results. Therefore positive and negative controls should always be run simultaneously with all specimens in each staining run.
The positive control permits the validation of appropriate processing of the sample. Tissues used as positive control should only be slightly positive. Thus, even a slight decrease in staining sensitivity will be uncovered.
Interpretation of staining results should only be done on intact cells since necrotic and degraded cells often show nonspecific
staining (10).
If the negative control is positive this points to non-specific staining. Regions or cell types which could function as an internal negative control (for example mammary ducts) can be found in most histological tissues.
Moreover, suitable procedures for quality control should be done with each new lot and after each methodical variation.

Expected results
During the reaction of the substrate with horseradish peroxidase in the presence of the chromogenic substrate DAB, a brown precipitate is formed at the location of the bound primary antibody. This reaction only takes place if the target antigen is present in the tissue.
The analysis is carried out by light microscopy.
For the determination of HER2 protein overexpression, only the cytoplasmic membrane staining pattern should be evaluated. The evaluation has to be performed by an experienced pathologist.
The German "S3 Guideline for diagnosis, therapy and aftercare of breast carcinoma" (S3 Leitlinie für die Diagnostik, Therapie und Nachsorge des Mammakarzinoms, 1. Aktualisierung 2008) recommends the following evaluation process (11):

A. positive HER2 Status:
ICH-Score 3+ : complete strong circular membrane reaction in more than 30% of the invasice tumour cells.

B. dubious HER2 Status:
ICH-Score 2+ : incomplete or weak circular membrane reaction in more than 10 % of invasive tumour cells or strong circular membrane reaction < 30 % of invasive tumour cells

C. negative HER2 Status:
ICH-Score 0 or 1 + : no membrane reaction or weak incomplete membrane reaction

Limitations of the procedure
Immunohistochemistry is a complex method in which histological as well as immunological detection methods are combined. Tissue processing and handling prior to immunostaining, for example variations in fixation and embedding or the inherent nature of the tissue can cause inconsistent results (12).
Endogenous peroxidase or pseudoperoxidase activity may cause non-specific staining. The enzyme activity is blocked by incubation with hydrogen peroxide solution.
Tissues containing Hepatitis B Surface Antigen (HBsAg) may give false positive results with HRP (horseradish peroxidase) detection systems (13).
Inadequate counterstaining and mounting can influence the interpretation of the results. The colour intensity of the reaction product can decrease with time, especially when exposed to light.
Interpretation of the results should always be done in context with tissue morphology and clinical report. The interpretation should be completed by appropriate controls and further diagnostic tests. Samples have to be examined by a qualified pathologist who is also responsible for choosing appropriate controls.
HER2*easy* Kit is only suitable and established for samples fixed in neutrally buffered formalin.
Biosite Histo Systems warrants that the product will meet all requirements described from its shipping date until the expiry date is reached, if the product is stored and utilised as specified. No additional guarantees can be given. Under no circumstances shall Biosite Histo Systems be liable for any damages arising out of the use of the reagent provided.

Troubleshooting
If unexpected staining or other deviations from the expected results are observed, which could possibly be caused by the reagents, please read the instructions below. If these are not of immediate help then please contact Biosite Histo Systems' technical support or contact your local distributor.

No staining on an actually positive control slide:

1. 2. Chromogenic substrate solution was too old.
2. The antigen/epitope was not stable in the fixation and/or pre-treatment procedure used. Try another fixation or
3. pre-treatment.

Weak staining:

1. Inadequate fixation or overfixation.
2. Incomplete dewaxing.
3. Too much wash buffer remains on the slides after washing, diluting the reagents applied in the next step.
4. Incubation times were too short.
5. Chromogenic substrate solution was too old.

Nonspecific background staining or overstaining:

1. Incomplete deparaffinisation.
1. Excessive tissue adhesive on slides.
2. Insufficient washing especially after the incubation with the enzyme polymer or the chromogenic substrate
3. solution. These washings are critical.
4. Tissue was allowed to (partially) dry out with reagents on.
5. Incubation times of certain reagents were too long.
6. The substrate is metabolised by endogenous horse radish peroxidase in the tissue. Maybe the hydrogen
7. peroxide solution used for blocking was inactivated.
8. Incubation times of the chromogenic substrate solution was too long or reaction temperature too high (e.g. if
9. temperature in the laboratory is high).

Performance characteristics
HER2*easy* Kit IHC was designed for the semi-quantitative detection of HER2 protein expression in formalin-fixed, paraffin-embedded tissue sections under standardised and reproducible conditions. It is not intended to provide patients or physicians with prognostic information.
The primary antibody clone CB11 against HER2/neu protein included in this HER2*easy* Kit IHC is a well established antibody. It has been compared with other immunohistochemical and molecular biological methods in a large number of studies (14-17).
The reagents of HER2*easy* Kit IHC have been evaluated in internal investigations. Both pre-treatment procedures described in this instruction (Procedure I and II) lead to identical results. Stained slides pre-treated according to procedure I of this package insert were externally evaluated in interlaboratory studies in 2007, 2008, and 2009 (Ringversuch HER2/neu - Immunhistochemie - der Qualitätssicherungs-Initiative Pathologie QuIP). In all cases the tests were passed and certified.

Bibliography

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