VITRO S.A
Calle Luís Fuentes Bejarano 60
Edificio Nudo Norte Local 3
41020 Sevilla (España)
F: +34 954 922 892
T: +34 954 933 200
www.vitro.bio

HER2/CEN 17 Dual CISH detection kit (Zytovision technology)

Complete kit for HER2 gene status evaluation by cromogenic in situ hybridization

DESCRIPTION:

This kit contains reagents to perform manual or automated chromogenic in situ hybridization (CISH) technique on human tissue sections fixed in buffered formalin and embedded in paraffin in order to determine the HER2 gene status in relation to the chromosome 17 centromere.
This kit contains all the solutions in sufficient quantity to perform 40 determinations following the protocol indicated.

Presentation: The general reference / presentation for this kit are:

MAD-001896QK-40	**40 tests**

This reference is for presentation packaging in Low Density Polyethylene (LDPE) dropper. In case the user wants other presentations (references / different volumes) must contact the supplier.

Intended Use IVD: Diagnostic in vitro in humans
Storage conditions: refrigerator between 2 and 8 ° C.
Warranty: The container once opened the reagent can be used until the expiration date indicated on the label. If the reagent has been stored under conditions other than those indicated in this document, the user must previously check its correct functionality considering that the product's warranty is no longer valid.
Special Handling Instructions: This reagent is specially designed for handling in Lab Vision Autostainer 480 and 780.
Warnings and Precautions: 1) The product may only be operated by trained users and authorized laboratories.
2) Please note that the ultimate responsibility in the optimization and interpretation of chromogenic hybridizations technique corresponds to the attending physician and technicians who use the kit. Also, this set of reagents is only a useful tool for the interpretation of morphological findings of each case in conjunction with other relevant diagnostic tests and patient´s clinical data.
3) The reagent contains sodium azide (NaN3) as a preservative. Although this product is highly toxic and if mixed with water or acids, mainly in the presence of metals there is danger of explosion, these risks are minimized to the maximum when used at concentrations below 0.05% as in this case. However, for handling this reagent the following precautions should be taken: a) Use of gloves and protective equipment established for hybridization and immunohistochemical techniques and lab strict compliance with the general safety practices existing in it; b) Do not store reagents in metal packaging and do not use metal tools for its handling c) Store waste for disposal in appropriate containers regulated under current regulations in each laboratory.

SPECIFICITY

The HER2 receptor, also known as human epidermal growth factor 2 neu, ERBB2 or CD340, is a proto-oncogene located on human chromosome 17, band 21, which encodes a transmembrane glycoprotein (p185) with tyrosine kinase activity. By their interaction and similarity with other members of the EGFR family of receptors for epidermal growth factor, HER2 is involved in cell differentiation during embryogenesis, following interaction between mesenchyme-epithelium-neuroectoderm and also influences the migration, differentiation and interaction of numerous cells.
HER2 overexpression has been demonstrated in over 20% of adenocarcinomas with different locations, including lung, uterus, gastrointestinal and urinary tract, breast and skin appendages.

In the case of breast and stomach overexpression of this oncoprotein, which is associated with poor prognosis, it relates to gene amplification and its secondary response to anti HER2 trastuzumab (Herceptin), a humanized monoclonal antibody capable of blocking this growth receptor. Similar effects have been obtained in other malignancies such as ovarian carcinomas, stomach and salivary gland.
The supplied probe containing polynucleotides labeled with digoxigenin directed to the target sequences of the HER2 gene and polynucleotides labeled with DNP for chromosome 17 alpha-satellite. The reaction can be visualized using primary antibodies, which are detected using secondary antibodies carrying polymer conjugated enzyme. Thus, the enzymatic reactions with their corresponding substrates leads to the formation of strong permanent red and green signals that can be visualized by light microscopy using a dry lens 40 or 60x.

DIAGNOSTIC APPLICATION AND ADVANTAGES:

Studies using DNA arrays evaluating the presence of hormone receptors and overexpression of HER2 gene divided breast carcinomas in 5 molecular subtypes: luminal A (ER +, HER2-), Luminal B (ER +, HER2 +), HER2 positive (ER-, HER2 +), normal breast type (ER +, PR +, HER2-) and basal-like (ER-, HER2-, EGFR + or keratin 5/6 +). For these reasons, the evaluation of HER2 has become an essential examination for indicating the proper treatment of breast carcinomas.
Most laboratories in North America and Europe use immunohistochemistry to determine HER2 status and in which equivocal (2 +) must be confirmed by fluorescence in situ hybridization (FISH). The immunohistochemical evaluation identifies the expression of the HER2 protein on the cell surface while FISH determines the actual degree of amplification of the HER2 gene. However both immunohistochemistry and FISH have some limitations. Thus, while immunohistochemistry is readily available in most laboratories the results are subject to changes in the methodology of obtaining and interpreting the immunohistochemical reaction between laboratories. FISH is a highly sensitive and specific method for determining HER2 gene amplification method which however, is more expensive than immunohistochemistry and requires special and expensive equipment and trained personnel which are not widely available in the diagnostic laboratories. In addition, evaluation of tumor morphology in FISH is not optimal, so it is difficult to distinguish between invasive carcinoma and in situ carcinoma. FISH is also limited by the fact that the fluorescent signal vanishes quickly at room temperature, making it difficult to conserve for future reference preparations.
The chromogenic in situ hybridization (CISH) is an alternative method for assessing the status of HER2 gene amplification using a chromogenic peroxidase-based reaction, which can be observed within a bright field microscope. As FISH, CISH determines the actual degree of HER2 gene amplification. However, unlike the first, positive signals can be identified using standard equipment in any laboratory. Furthermore, the histopathology of the specimen can be evaluated at the same time and the signal does not disappear and remains stable over a long period of time, enabling the storage of samples at room temperature. For all these reasons CISH has greater potential for integration into routine diagnostic laboratories than FISH.
Patients with tumors with 3 + staining by immunohistochemistry or positive HER2 FISH are considered positive and therefore are eligible for treatment with Herceptin, while patients with tumors 2 + in immunohistochemistry should be evaluated to confirm HER2 gene amplification by FISH or CISH. Also, according to the latest recommendations of the American Society of Clinical Oncology and the College of American Pathologists, CISH is also indicated in cases of breast carcinomas grade 1 ductal type and hormone receptor-positive lobular and tubular carcinomas, mucinous, cribriform, adenoid cystic and triple negative cases which demonstrated a 3 + positive immunohistochemical initial results. If immunostaining was negative in a tumor biopsy, CISH may be also preferred for evaluating the tumor resection specimen in cases of invasive carcinoma grade 3, in invasive carcinomas with very little representation in the biopsy, in resected specimens of high grade carcinomas other than those diagnosed in a previous biopsy or in cases with preanalytical technical artifacts.

INTERPRETACIÓN

The CISH hybridization signal for a single copy of HER2 gene appears as a distinctive mark, as a dark green dot while the signal from the centromeric region of chromosome 17 as a punctate signal of bright red color, which can be distinguished clearly by the background counterstaining with hematoxylin. The visualization of the signals must be made with an objective of at least 40x.
No filter lens to enhance the contrast should be used as this may distort the color signal. For brightly colored signals is desirable to increase the diaphragm aperture. Also make sure to focus up and down when doing a core assessment as the red and green signals may be located in different planes.

Prior to quantify the signal, the tissue section / extended cells must be scanned with an objective of 10x or 20x to detect any possible intratumoral heterogeneity. If heterogeneity exists, for the evaluation should be chosen a representative area for the amplification status of the tumor. For the numerical quantification of the signals, necrotic areas, overlapped nuclei and weak signal intensity should be avoided.
In *normal diploid nuclei* without amplification of the gene, even in normal cells or other accompanying cells, in each nucleus two punctate green and red signals with smooth contour are visible (see Fig. 1). Due to mitosis, additional signals might be seen even in a small percentage of non-neoplastic cells. Occasionally, because the signals might be located above or below the plane of the section, cells with no signal could be seen.
If *aneuploidy of chromosome 17* (see fig. 2) multiple additional red signals will be visible.
If *HER2 gene amplification* (see Fig. 3) the specific green HER2 gene signals are visible as multiple points or small groups of irregular shape. As a reference for the green signal, normal cells present in the same preparation should only be used. In addition, red centromere signals will be clearly visible.
In case of *high gene amplification* many green dots or large groups will be visible in the nuclei (see fig.4). As a reference for the green signal, normal cells present in the same preparation should only be used. Red centromeric signals may be superimposed and may not be apparent or visible.

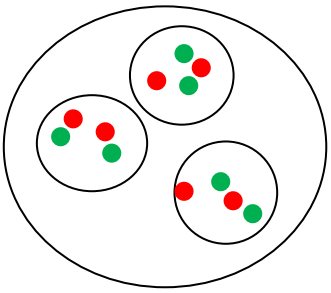

Fig.1. Normal cells

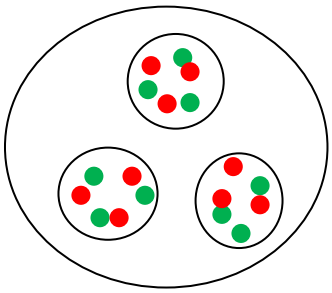

Fig.2. Chromosome 17 aneuploidy

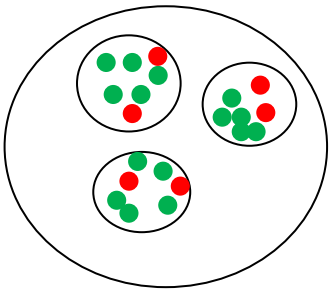

Fig.3. Low amplification of the gene

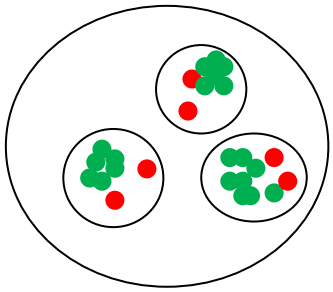

Fig.4. High amplification of the gene

However for the correct interpretation of the results is recommended to follow the latest guidelines for effective assessment for each type of tissue used in the published literature.

LIMITATIONS OF REACTIVE

The use of frozen tissue has not been evaluated.

SAMPLE TYPES

Sections of 4 microns thick mounted on special slides for immunohistochemistry and obtained from paraffin-embedded tissues, preferably fixed in buffered formalin.

PRINCIPLE OF ANALYTICAL METHOD (in situ hybridization)

In situ hybridization (ISH) allows the detection of specific sequences of DNA or RNA in histological and cytological samples without losing morphological details. The ISH technique is based on hybridization between a DNA or RNA sequence specifically labelled (probe) and a sequence of DNA or RNA present in the sample. If there is any complementarity between the sequences the hybridization occurs, which results in a hybrid product. Hybrids can be easily viewed by a chromogenic immunohistochemical staining procedure (CISH) directed toward specific marker of the probe employed.
The ISH technique is highly sensitive, specific and easy to perform, and in addition, contains no radioactive products. The reagents supplied in this kit are perfectly matched and therefore, the kit is an easy to use tool to perform CISH.

COMPONENTS AND REAGENTS INCLUDED IN THE KIT			
EDTA Buffer 10X pH8 (PT Module)	MAD-004072R/D	40 tests	1000 ml
Pepsin Solution (Ready to use)	MAD-024080R-4	40 tests	6 ml
Peroxidase Blocking Reagent	MAD-021540Q-4	40 tests	6 ml
HER2/CEN 17 CISH PROBE	MAD-001896HS-40	40 tests	400µl
Post-Hybridization Wash Buffer (Ready to use)	MAD-FS0105-1	40 tests	1000 ml
ANTI-DIG/ANTI-DNP MIX	MAD-001896QK-1B40	40 tests	4 ml
HRP/AP-POLYMER-MIX	MAD-001896QK-2B40	40 tests	4 ml
AP-RED CHROMOGEN			
AP RED SOLUTION A	MAD-001896QK-3.1A40	40 tests	0,4 ml
AP RED SOLUTION B	MAD-001896QK-3.1B40	40 tests	15 ml
HRP-GREEN CHROMOGEN			
HRP GREEN SOLUTION A	MAD-001896QK-4.1A40	40 tests	0,8 ml
HRP GREEN SOLUTION B	MAD-001896QK-4.1B40	40 tests	15 ml
Hematoxilina de Contraste HDH3	MAD-HDH3-4	40 tests	5 ml

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED IN THE KIT

- Humid incubation chamber
- Silane coated or electrically charged slides
- Coverslips
- Thermal plate or oven (37 ° C)
- Dewaxing and hydratation battery (xylene, absolute ethanol and of 80% and 70% concentrations
- TBS buffer
- Micropipettes
- Optical microscope

SAFETY RECOMMENDATIONS

This product is intended for laboratory professional use only. The product is NOT intended to be used as a drug or for domestic purposes. The current version of the Safety Data Sheet for this product can be downloaded by searching the reference number at www.vitro.bio or can be requested at regulatory@vitro.bio.

PROTOCOLE FOR CISH C-ERB-B2 ON BUFFERED FORMALIN FIXED AND PARAPHIN EMBEDED BIOPSIES USING VITRO S.A KIT

This protocol is recommended for performing CISH on tissue sections fixed in buffered formalin and embedded in paraffin.

1. Dewaxing

a. Incubate slides with paraffin tissue sections in the oven at 60 ° C overnight.
b. Dewaxing:
   - Xylene 10 min 2x
   - Absolute ethanol 2x 5 min

- Ethanol 80% 5 min
- Ethanol 70% 5 min
- Distilled water (10 min)

2. Temperature pretreatment

a. Place the slides in the PT module with an EDTA pH8 buffer solution for 20 min at 95 ° C or immersed the preparations in a coplin jar with EDTA buffer pH8 and place them in a water bath, 20 min at 95 ° C.
b. Wash 3 times with TBS buffer at RT.
c. Air dry sections

3. Enzymatic digestion

a. Apply **100ul** of **ready-to-use pepsin solution** (which should be brought to RT before use) onto the tissue and incubate **7 min** a 37ºC.
Note: the optimal incubation time for some section may be shorter or longer, depending on size, tissue preservation and heterogeneous cell content

b. Wash 3 times with TBS buffer at RT - Tween 20 at RT.
c. Dehydrate the preparation in crescent alcohol solutions:
- Ethanol 70 % 3 min
- Ethanol 80% 3 min
- Absolute ethanol 3 min
- Air dry sections

4. Denaturation and Hybridization

a. **Apply** 10 µl of **HER2/CEN17 CISH** probe over the tissue.
b. **Place** a coverslip on the section and sealed them with rubber cement.
c. Proceed with the **denaturation** on a hot plate for **5 min** at **80º C** and continue by incubating in humid chamber **16 hours** at **37º C** for the hybridization step.

5. Post-hybridization washes

a. Remove the coverslips by immersing the slides in **post-hybridization wash buffer** for **5min at RT.**
b. Wash the slides with preheated **post-hybridization wash buffer 5 min at 75º C**. (Is recommended not to wash more than 5-6 slides simultaneously).
c. Maintain the slides in TBS solution until detection steps.

6. Detection and visualization (manual)

a. Apply **100 µl of peroxidase blocking solution** on the tissue and incubate 10 min at RT
b. Wash 3 times in TBS – Tween 20.
c. Apply **100 µl of MIX of Secondary Antibody** and incubate 20 min at RT.
d. Wash 3 times in TBS – Tween 20.
e. Apply **100 µl of Mix of Polymers** and incubate 20 min at RT.
f. Wash 3 times in TBS – Tween 20.
g. Apply **100 µl of Red Chromogen** and incubate 10 min at RT. (the final solution should be prepare by mixing 1000µl of substrate buffer with 20µl of Red chromogen (one drop)).
h. Wash 3 times with **Distilled Water**
i. Apply **100 µl of Green Chromogen** and incubate 10 min at RT. (the final solution should be prepare by mixing 1000µl of substrate buffer with 40µl of Green chromogen (two drops)).
j. Wash 3 times with **Distilled Water**

7. Contrast staining and mounting

a. Stain with **100ul of contrast haematoxylin solution for 30 sec.**

b. **Bluing** in tap water for **5 min**.
c. **Dehydrate and clear** with increasing concentrations of alcohols (30sec) and xylene (30sec).
d. **Mount** and interpret the results under a microscope.

INCIDENTS AND COMPLAINS

It is recommended to thoroughly follow all instructions contained in these technical data sheet. In case of occurrence of atypical or unexpected results please contact the Vitro SA sales representative of the area. If not, please contact Vitro S.A using its contact information as mentioned above.

INCIDENTS	CAUSES	MEASURES
Poor morphology	Excess of enzymatic digestion (hollows in the tissue or a "ghosty" appearance)	Reduce the incubation time with the pepsin
No or weak intensity signals	Improper fixation	Optimize the fixation time; check the quality of the fixation solution and its compatibility with the ISH technique.
	Excess or insufficient enzymatic digestion	Optimize the time for enzymatic treatment
	Inadequate temperature for denaturalization and/or hybridization, of the post hybridization solution or of the probe	Check and adjust the temperature if necessary
	Short hybridization	Hybridize at least 12 hours and extend the time if necessary
	Short incubation with the cromogen	Extend the time of incubation
	Chromogen solution prepared to early	Prepare the chromogen solution before its use
	Strong counterstaining	Reduce the time of incubation with de haematoxylin
	Chromogen solutions incorrectly prepared	Revise the quantities used and stick to the recommendations
Weak red signals	The red chromogen solution has been exposed to direct light	Do not prepare and do not use the chromogen solution under bright light
Weak green signals	To large post staining wash	Respect the recommended times
	To large counterstaining	Reduce the time for the counterstaining
Less defined or fused green signals	Inadequate mounting medium	Use a xylene based mounting medium
	The sections were not correctly dehydrated	Use fresh solutions of alcohol and xylene
Weak staining focally	Incomplete dewaxing	Use fresh solutions and check the time of dewaxing
	Low volume of dewaxing solution	Be sure to use adequate solution of dewaxing in order to cover the whole slide
	Air bubbles trapped before the hybridization or during mounting	Avoid generating bubbles
Strong background staining	Insufficient staining	Use fresh solutions of buffer or deionized or distilled water as frequent as necessary

	The sections dried during hybridization	Use humid chambers for hybridization and seal well the coverslips
	To low post-hybridization washing temperature	Check and adjust the temperature if necessary

LIMITATIONS OF THE REACTIVE

If you have met all the conditions of storage and handling in the laboratory, this reagent is guaranteed throughout its warranty life. Vitro S.A is not responsible for damage, personal injury or economic loss that this reagent can be involved.

REFERENCES:

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LABEL AND BOX SYMBOLS

Explanation of the symbols of the product label and box:

IVD	Health product for in vitro diagnosis.		Expiration date
REF	Catalog number		Temperature limit
LOT	Lot code		Manufacturer
	Refer to the instructions of use		Sufficient content for <n> assays
	Material safety data sheet		