IMPATH HER2 FISH

Product Identification

Cat. No.	Description
47862	ImPath HER2 FISH

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Distributed by:
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CE IVD
In vitro diagnostic medical device
according to EU directive 98/79/EC

Intended Use

The ImPath HER2 FISH (cat. no. 47862) is intended to be used in combination with the ImPath ISH Detection Kit (cat. no. 44996) for the detection of the human ERBB2 gene (a.k.a. HER2) as well as chromosome 17 alpha-satellites in formalin-fixed, paraffin-embedded tissue or cell samples by fluorescence *in situ* hybridization (FISH) on the ImPath 36 (cat. no. 43965).
Interpretation of results must be made within the context of the patient's clinical history with respect to further clinical and pathologic data of the patient by a qualified pathologist.

Summary and Explanation

The ERBB2 gene (a.k.a. HER2 and NEU) is located in the chromosomal region 17q12 and encodes the cellular growth factor receptor p185.

Amplification of the proto-oncogene ERBB2, observed in approximately 20% of all breast cancer samples, has been correlated with a poor prognosis of the disease. Similar results have been obtained for a variety of other malignant neoplasms e.g. ovarian cancer, stomach cancer, and carcinomas of the salivary gland.

Principles and Procedures

The presence of certain nucleic acid sequences in cells or tissues can be detected by fluorescence *in situ* hybridization (FISH) using DNA probes labeled with fluorescent dyes. The hybridization results in duplex formation of sequences present in the test object and the specific probe which can be visualized using fluorescence microscopy, employing suitable filters.
The ImPath HER2 FISH contains orange-labeled polynucleotides (excitation at 547 nm and emission at 572 nm, similar to rhodamine), which target the ERBB2 gene, and green-labeled polynucleotides (excitation at 503 nm and emission at 528 nm, similar to FITC), which target alpha-satellite-sequences of the centromere of chromosome 17 (D17Z1).

Materials and Methods

Reagents Provided

The stated product is a ready-to-use FISH probe in a vial made for the use with the ImPath 36. The vial is equipped with an RFID tag that is read by the ImPath 36 to provide product and lot specific information.

Reconstitution, Mixing, Dilution

The product is ready-to-use. No reconstitution, mixing, or dilution is required. Differences in tissue processing and technical procedures in the laboratory may produce significant variability in results and consequently require regular use of controls (see Quality Control Procedures section).

Materials and Reagents Needed but Not Provided

The following reagents and materials may be required for staining, but are not provided with the FISH probe.

1. Positive and negative control tissue
2. Microscope slides, positively charged
3. Drying oven capable of maintaining a temperature of 50-60°C
4. Staining jars or baths
5. Timer
6. Ethanol or reagent alcohol
7. ImPath ISH Detection Kit (cat. no. 44996)
8. DAPI/Antifade*
9. Cover glass
10. Fluorescence microscope (400-1000x)
11. Appropriate filter sets

*Recommended for use: ImPath DAPI (cat. no. 47861)

Storage and Handling

Store at 2-8˚C in an upright position. Store protected from light.
Prior to opening the vial, shake down liquid.
To ensure proper reagent delivery and stability of the FISH probe, the reagent must be returned to the storage conditions identified above immediately after use.
When properly stored, the reagent is stable until the date indicated on the label. Do not use reagent beyond this expiration date for the prescribed storage method.

Specimen Collection and Preparation for Analysis

Routinely processed, neutral-buffered formalin-fixed, paraffin-embedded, tissues are suitable for use with this FISH probe. The recommended tissue fixative is 10% neutral-buffered formalin.
Each section should be cut to the appropriate thickness (approximately 3-5 µm) and placed on a positively charged glass slide. Slides containing the tissue section may be baked for at least 2 hours (but not longer than 16 hours) in a 50-60°C oven.

Warnings and Precautions

1. Take reasonable precautions when handling reagents. Use disposable gloves and lab coats when handling suspected carcinogens or toxic materials.
2. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
3. Tissue and cell specimens and all materials contacting them should be handled as biohazardous materials and disposed of with proper precautions. Never pipette by mouth.
4. Avoid microbial contamination of reagents, as this could produce incorrect results.
5. The user must optimize Pepsin solution incubation times and temperatures.
6. The prediluted, ready-to-use reagent is optimally diluted, and further dilution may result in loss of staining quality.
7. This product is classified as a hazardous substance. For further details please see corresponding material safety data sheet.
8. The user must validate any storage conditions other than those specified in the package insert.
9. As with any product derived from biological sources, proper handling procedures should be used.

Instruction for Use

The ImPath HER2 FISH (cat. no. 47862) is intended to be used in combination with the ImPath ISH Detection Kit (cat. no. 44996) on the ImPath 36 (cat. no. 43965).

ImPath ISH Protocol:
ImPath ISH Detection Kit (cat. no.: 44996)

Protocol Steps:
Step By Step Procedure

1. Follow the ImPath 36 instructions for use to setup the reagent for use on the instrument.
2. Load slides, FISH probe, and ImPath ISH Detection Kit onto the ImPath 36 according to the ImPath 36 instructions for use.

   Set Pepsin digestion time according to conditions prevalidated by the user.
3. Start the run.
4. When the staining run is complete, remove slides from instrument, dehydrate with 70 %, 90 %, and 100 % ethanol each for 1 min.
5. Air dry samples in the dark.
6. Mount with a DAPI/Antifade solution (it is recommended to use ImPath DAPI (cat. no. 47861)) and incubate in the dark for 15 min.
7. Cover with coverslip and store the slides in the dark at 2-8°C.

Quality Control Procedures

Positive Tissue Control
A positive tissue control must always be run with every staining procedure performed. This tissue may contain both, amplified (positive) and non-amplified (negative) staining cells, and serves as both the positive and negative control tissue.
Known positive tissue controls should be utilized for monitoring the correct performance of processed tissues and test reagents, not as an aid in determining a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate appropriate positive staining, results with the patient specimens must be considered invalid.

Negative Tissue Control
The same tissue used for the positive tissue control may be used for the negative tissue control.
Non-neoplastic cells on the slide/in the tumor section such as e.g. fibroblasts, epithelial cells, and/or lymphocytes serve as internal control and have to exhibit the expected normal signal pattern and can therefore serve as negative tissue control. If these cells fail to demonstrate appropriate staining, results with the repsective specimen must be considered invalid.

Unexplained Discrepancies
Unexplained discrepancies in controls should be referred to A.Menarini Diagnostics Customer Service immediately. If quality control results do not meet specifications, patient results are invalid. See the Troubleshooting section of this insert. Identify and fix the problem, then repeat the entire procedure with the patient samples.

Interpretation of Results

With the use of appropriate filter sets, the hybridization signals of labeled ERBB2 gene appear orange, the hybridization signals of labeled alpha-satellite-sequences of the centromere of chromosome 17 appear green. In interphases of normal cells or cells without aberrations of chromosome 17, two ERBB2 signals and two chromosome 17 signals appear. In cells with a gene amplification, an increased number of gene specific signals or signal clusters are visible.
Care should be taken not to evaluate overlapping cells, in order to avoid false results, e.g. an amplification of genes. Due to decondensed chromatin, single FISH signals can appear as small signal clusters. Thus, two or three signals of the same size, separated by a distance equal to or less than the diameter of one signal, should be counted as one signal.
Tissue artifacts like tissue at the boundary or retracted or squeezed tissue should be excluded from evaluation. Do not evaluate patient tissue if controls are not as expected. Reject the object if it shows strong autofluorescence. Over-digestion can be recognized by dark areas visible inside of the nuclei and should be excluded from evaluation.
A negative or unspecific result can be caused by multiple factors (see Troubleshooting chapter of this insert).

Limitations

1. This reagent is “for professional use only” as fluorescence *in situ* hybridization is a multiple step process that requires specialized training in the selection of the appropriate reagents, tissues, fixation, processing, preparation of the FISH slide, and interpretation of the staining results.
2. For laboratory use only.
3. For in vitro diagnostic use.
4. Tissue staining, especially signal intensity and background staining, is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts or false results. Inconsistent results may result from variations in fixation and embedding methods, as well as from inherent irregularities within the tissue.
5. Excessive or incomplete counterstaining may compromise proper interpretation of results.
6. The quality of the signals depends on the right positioning of the tissue on the lower half of the slide. For further information on proper placement of the tissue please contact your sales representative of A.Menarini Diagnostics.
7. The clinical interpretation of any positive staining, or its absence, must be done within the context of clinical history, morphology, other histopathological criteria as well as other diagnostic tests. It is the responsibility of a qualified pathologist to be familiar with the FISH probes, reagents, diagnostic panels, and methods used to produce the stained preparation. Staining must be performed in a certified, licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
8. Ready-to-use FISH probes and reagents are provided at optimal dilution for use as instructed. Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users in any circumstance must accept responsibility for the interpretation of patient results.

9. Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated because of biological variabilities in neoplasms or other pathological tissues. Contact A.Menarini Diagnostics customer service with any suspected, documented unexpected reactions.

Expected Results

The following table demonstrates the performance of the ImPath HER2 FISH probe compared with a CE certified manual HER2 FISH probe on formalin-fixed, paraffin-embedded mammary carcinoma tissues. According to ASCO Guideline (2013) a HER2/CEN 17 ratio ≥2.0 is considered positive.

		ImPath HER2 FISH		
		negative (HER2/CEN 17 ratio <2.0)	positive (HER2/CEN 17 ratio ≥2.0)	total
reference	negative (HER2/CEN 17 ratio <2.0)	14	0	14
	positive (HER2/CEN 17 ratio ≥2.0)	0	5	5
	total	14	5	19

A high concordance of 100%, specificity of 100% and sensitivity of 100% on mammary carcinoma tissue is given for the ImPath HER2 FISH probe when performed on the ImPath 36.

Troubleshooting

1. If weak or no signals can be observed, proteolytic pretreatment may not have been carried out properly and the Pepsin incubation time should be optimized.
2. Furthermore, a too low concentrated wash buffer can lead to weak signals. The concentration of the wash buffer should consequently be checked.
3. Further reasons for weak signal intensities may be a wrongly adjusted fluorescence microscope. Make sure to use a properly configured and well-maintained fluorescence microscope with appropriate filter sets.
4. Too strong beam of light while handling the probe/slides, may also result in weak or no signals. Handling of the probe and of the stained slides should be accomplished protected from direct sunlight.
5. If cross hybridization signals or strong background staining occur, proteolytic pretreatment may have been too strong and the Pepsin incubation time should be optimized.
6. Furthermore, a too high concentrated wash buffer can lead to cross hybridization or strong background staining. The concentration of the wash buffer should consequently be checked.
7. If tissue sections wash off the slide, slides should be checked to ensure that they are positively charged. Other possibilities that could have adverse effect on tissue adhesion include insufficient drying of the tissue section on the slide prior to staining or fixation in formalin that was not properly neutral-buffered. Tissue thickness may also be a contributing factor.

For corrective action, refer to the Step by Step Procedure section or contact A.Menarini Diagnostics customer service.

References

1 Kievits T, *et al*. Rapid subchromosomal localization of cosmids by nonradioactive in situ hybridization. Cytogenet Cell Genet 53: 134-6. (1990)

2 Wilkinson DG: In Situ Hybridization, A Practical Approach, Oxford University Press ISBN 0 19 963327 4. (1992)

3 Wolff AC, *et al*. Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update. J Clin Oncol. 31(31):3997-4013 (2013)