HER2 Dual ISH 3-in-1 Xenograft Slides

REF 783-4422

05640300001

IVD

INTENDED USE

HER2 Dual ISH 3-in-1 Xenograft Slides are intended for initial assay installation and/or troubleshooting activities with the INFORM HER2 Dual ISH DNA Probe Cocktail or VENTANA HER2 Dual ISH DNA Probe Cocktail on BenchMark IHC/ISH instruments.

This product is intended for in vitro diagnostic (IVD) use.

SUMMARY AND EXPLANATION

HER2 Dual ISH 3-in-1 Xenograft Slides may be useful for a preliminary validation of the processing method used for staining slides with INFORM HER2 Dual ISH DNA Probe Cocktail or VENTANA HER2 Dual ISH DNA Probe Cocktail and in troubleshooting activities in the event of tissue failures. Each slide contains three xenograft sections that are formalin-fixed and embedded in a single paraffin block. The xenografts have been selected based upon characterization established in published literature for protein over-expression as well as gene copy number. The use of these xenograft tissue slides is advantageous because they are well characterized for HER2 and Chromosome 17 copy numbers and are processed pre-analytically in a controlled manner. HER2 Dual ISH 3-in-1 Xenograft Slides are sold separately from the probe and detection kits, and are not required for routine use with the assay. Since every normal cell contains two copies of HER2 and Chromosome 17, there is no true negative specimen control. Figure 1 illustrates the layout of the xenograft slides. Table 1 lists the gene status of the xenograft slides.

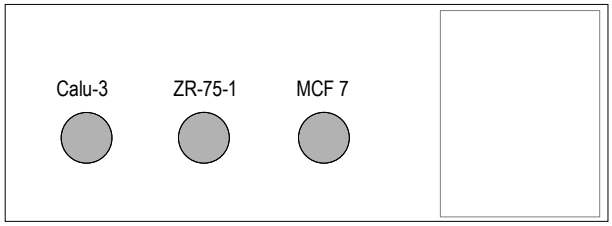


Figure 1. Schematic Layout of Xenograft Slide.

Table 1. Characterization of Xenograft Slide.

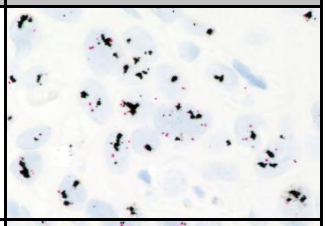
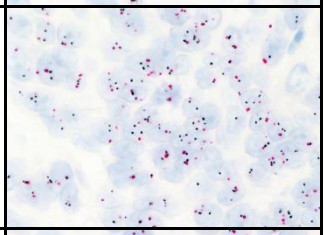
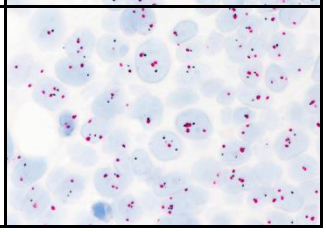
Cell Line	HER2 Protein Expression Level	Approximate HER2 Gene Copy # by FISH	Average HER2/Chr17 Ratio by FISH	Average HER2/Chr17 Ratio by DISH
Calu-3	3+	> 6 copies/nuclei	5.01	5.53
ZR-75-1	1+	3 copies/nuclei	1.36	1.34
MCF 7	0	1-2 copies/nuclei	1.0	0.85

PRINCIPLE OF THE PROCEDURE

In general, in situ hybridization (ISH) uses labeled probes to detect specific DNA or RNA sequences in fixed tissue. Target sequences are exposed by heating the tissue and probe solution to denature nucleic acids. The reaction is then cooled allowing the labeled nucleic acid probe to hybridize to its complementary nucleic acid sequence in the tissue.

HER2 Dual ISH 3-in-1 Xenograft Slides are for use with the INFORM HER2 Dual ISH DNA Probe Cocktail or VENTANA HER2 Dual ISH DNA Probe Cocktail, and BenchMark IHC/ISH instruments. The staining protocol consists of numerous steps in which reagents are incubated for pre-determined times at specific temperatures. At the end of each incubation step, the BenchMark IHC/ISH instrument washes the sections to remove unbound material and applies a liquid coverslip which minimizes the evaporation of the aqueous reagents from the slide. Results are interpreted using a light microscope. For more detailed information on instrument operation, refer to the appropriate User Guide. Table 2 illustrates the staining pattern of the xenograft slide.

Table 2. Characteristic Staining Pattern of Xenograft Slide.

Cell Line	VENTANA HER2 Dual ISH DNA Probe Cocktail
Calu-3	
ZR-75-1	
MCF 7	

MATERIAL PROVIDED

Each HER2 Dual ISH 3-in-1 Xenograft Slide contains three different human carcinoma cell lines generated as tumors (xenografts) in SCID (severe combined immunodeficiency) mice. The xenografts are formalin-fixed, paraffin-embedded, cut into 4 µm sections and placed on positively charged slides. Ten slides, each containing the three different xenografts in a single paraffin section, are provided.

Refer to the appropriate VENTANA detection kit method sheet for detailed descriptions of: Principle of the Procedure, Material and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Troubleshooting, Interpretation of Results, and Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, are not provided.

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. VENTANA HER2 Dual ISH DNA Probe Cocktail (Cat No. 760-6072 / 08314365001 or Cat. No. 800-6043 / 08314373001)
2. VENTANA Silver ISH DNP Detection Kit (Cat. No. 760-516 / 08318883001)
3. VENTANA Red ISH DIG Detection Kit (Cat. No. 760-512 / 08318832001)
4. INFORM HER2 Dual ISH DNA Probe Cocktail (Cat No. 780-4422 / 05586640001 or Cat. No. 800-4422 / 05899826001
5. *ultra*View SISH DNP Detection Kit (Cat. No. 800-098 / 05907136001)
6. *ultra*View Red ISH DIG Detection Kit (Cat. No. 800-505 / 05907128001)
7. HybReady Solution (Cat. No. 780-4409 / 05917557001)
8. *ultra*View Silver Wash II (Cat. No. 780-003 / 05446724001)
9. ISH Protease 3 (Cat. No. 780-4149 / 05273331001)
10. 10X SSC (Cat. No. 950-110 / 05353947001)
11. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
12. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)

13. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
14. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
15. Cell Conditioning Solution (CC2) (Cat. No. 950-123 / 05279798001)
16. ULTRA Cell Conditioning Solution (ULTRA CC2) (Cat. No. 950-223 / 05424542001)
17. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
18. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
19. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
20. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
21. BenchMark IHC/ISH instrument
22. General purpose laboratory equipment

STORAGE AND STABILITY

Store at 15-30°C. Do not freeze.

HER2 Dual ISH 3-in-1 Xenograft Slides are expiration dated. When properly stored, the slides are stable to the date indicated on the label. Do not use slides beyond the expiration date.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. **CAUTION:** In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Take reasonable precautions when handling glass slides. Use disposable gloves when handling suspected carcinogens or toxic materials (example: xylene or formamide).
5. Empty the waste container prior to starting a run. A run will not start unless the waste container is emptied prior to starting a run.
6. Materials of human or animal origin should be handled as potentially biohazardous and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[1,2]
7. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
8. Avoid microbial contamination of product, as this could produce incorrect results.
9. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and instructions for use of all necessary components, located at dialog.roche.com.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
12. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

STAINING PROCEDURE

Xenograft slides are for use on a BenchMark IHC/ISH instrument in combination with INFORM HER2 Dual ISH DNA Probe Cocktail or VENTANA HER2 Dual ISH DNA Probe Cocktail and accessories. Refer to the INFORM HER2 Dual ISH DNA Probe Cocktail method sheet or VENTANA HER2 Dual ISH DNA Probe Cocktail method sheet for slide processing, slide scoring, and slide dehydration procedures. The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument User Guide.

Table 3. Recommended staining protocols for HER2 Dual ISH 3-in-1 Xenograft Slides for INFORM HER2 Dual ISH DNA Probe Cocktail (P/N 780-4422) on BenchMark IHC/ISH instruments (US only).

Procedure Step	XT	ULTRA
Deparaffinization	Selected	Selected
Cell Conditioning	CC2, Mild - 8 min, Standard - 12 min, Extended - 8 min	ULTRA CC2, Standard
ISH-Protease 3	8 min	8 min
Denaturation	20 min	8 min
Hybridization	6 hours	6 hours
Stringency wash	76°C	72°C
SISH Multimer	16 min	Not selectable
Silver Chromogen	4 min	8 min
Red ISH Multimer	24 min	Not selectable
Red Chromogen	8 min	8 min
Counterstain	Hematoxylin II, 8 min	Hematoxylin II, 8 min
Post Counterstain	Bluing, 4 min	Bluing, 8 min

Table 4. Recommended staining protocols for HER2 Dual ISH 3-in-1 Xenograft Slides for INFORM HER2 Dual ISH DNA Probe Cocktail (P/N 800-4422) on BenchMark IHC/ISH instruments.

Procedure Step	GX	XT	ULTRA
Baking Temperature	N/A	N/A	Selected, 63ºC
Baking Time	N/A	N/A	20 min
Deparaffinization	Selected	Selected	Selected, 72ºC
Extended Deparaffinization*	Not Selected	Not Selected	Not Selected
Cell Conditioning	CC2, Mild- 8 min, Standard, - 12 min, Extended - 8 min	CC2, Mild - 8 min, Standard – 12 min, Extended – 8 min	ULTRA CC2, 86 ºC, Mild - 8 min, Standard - 12 min, Extended - 8 min
ISH-Protease 3	8 min	8 min	8 min
Denaturation	20 min	20 min	20 min
Hybridization	6 hours	6 hours	6 hours
Stringency wash	76°C	76°C	76°C
SISH Multimer	16 min	16 min	32 min
Silver Chromogen	4 min	4 min	4 min
Red ISH Multimer	24 min	24 min	24 min
Red Chromogen	8 min	8 min	8 min
Counterstain	Hematoxylin II, 8 min		
Post Counterstain	Bluing, 4 min		

* The Extended Deparaffinization option is intended to mitigate severe nuclear bubbling due to excess paraffin.

Table 5. Recommended staining protocol for HER2 Dual ISH 3-in-1 Xenograft Slides for VENTANA HER2 Dual ISH DNA Probe Cocktail on BenchMark IHC/ISH instruments.

Procedure Step	GX	XT	ULTRA
Baking	Not Selected	Not Selected	Not Selected
Cell Conditioning	CC1, 20 min	CC1, 20 min	ULTRA CC1, 16 min
Cell Conditioning	CC2, 20 min	CC2, 20 min	ULTRA CC2, 16 min

Procedure Step	GX	XT	ULTRA
ISH-Protease 3	8 min	8 min	8 min
Stringency Wash Temp	78°C	78°C	76°C

QUALITY CONTROL PROCEDURE

Positive and Negative Sample Controls

HER2 Dual ISH 3-in-1 Xenograft Slides should be run during preliminary assay validation and/or troubleshooting activities as an aid in the assessment of assay performance on known positive samples. The slides should be utilized only 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 internal tissue controls fail to demonstrate positive staining in the test specimen, results with the test specimens should be considered invalid.

Unexplained Discrepancies

Unexplained discrepancies in controls should be referred to your local support representative immediately. If quality control results do not meet specifications, patient results are invalid. Refer to the Troubleshooting section. Identify and correct the problem, then repeat the patient samples.

STAINING INTERPRETATION / EXPECTED RESULTS

The VENTANA automated ISH procedure causes a discrete silver and red reaction product to precipitate at the target sites localized by the probes. A qualified reader experienced in ISH procedures must evaluate controls and qualify the stained product before interpreting results.

As xenografts are comprised of human tumor cell lines grown in mice;, the non-tumor cells are mouse cells containing mouse HER2 and Chromosome 17 sequences which will not be detected by either probe.

When the system and reagents are functioning properly, the Calu-3 control is expected to have > 6 HER2 SISH signals in the viable tumor cells. The ZR-75-1 and MCF7 control areexpected to have 1-3 HER2 signals (Table 1).

LIMITATIONS

General Limitations

1. ISH is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, specimen selection, processing, preparation of the ISH slide, and interpretation of the results.
2. Excessive or incomplete counterstaining may compromise proper interpretation of results.
3. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history and morphology, and must be complemented by proper controls and other diagnostic tests. Staining must be performed under the supervision of a qualified reader who is responsible for reviewing the stained slides and ensuring the adequacy of controls.

SPECIFIC LIMITATIONS

HER2 Dual ISH 3-in-1 Xenograft Control Slides are to be used with INFORM HER2 Dual ISH DNA Probe Cocktail or VENTANA HER2 Dual ISH DNA Probe Cocktail to monitor the performance of the automated ISH staining assay during preliminary validation and/or troubleshooting activities. These xenograft slides may have been fixed or processed differently from the test specimen and, as such, provide control for all reagents and method steps except fixation and tissue processing.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

The performance of HER2 Dual ISH 3-in-1 Xenograft Slides was evaluated through reproducibility and other relevant studies in conjunction with INFORM HER2 Dual ISH DNA Probe Cocktail and VENTANA HER2 Dual ISH DNA Probe Cocktail.

1. Intra-run reproducibility staining of HER2 Dual ISH 3-in-1 Xenograft Slides was determined by testing 3 slides on Day 1 and 2 and 2 slides on Days 3-5 on BenchMark, BenchMark XT and BenchMark ULTRA instruments over 5 non-consecutive days using the INFORM HER2 Dual ISH DNA Probe Cocktail. Acceptable staining was found on all slides tested. Users should verify intra-run (within run) reproducibility results by staining several sets of serial sections with low, medium and high target sequences in a single run.
2. Inter-run reproducibility staining of HER2 Dual ISH 3-in-1 Xenograft Slides was determined by staining and analyzing 3 HER2 Dual ISH 3-in-1 Xenograft Slides on Day 1 and 2 and 2 slides on Day 3-5 over five non-consecutive days on six instruments (2 BenchMark, 2 BenchMark XT, 2 BenchMark ULTRA instruments) using the INFORM HER2 Dual ISH DNA Probe Cocktail. Acceptable staining was found on all slides tested. Users should verify inter run (between run) reproducibility results by staining several sets of serial sections with low, medium, and high target sequences on different days.
3. The human cell lines used in the HER2 Dual ISH 3-in-1 Xenograft Slides have been characterized by FISH using Abbott/Vysis PathVysion. The results obtained (Table 1) are consistent with data obtained from previous studies.3,4,5,6,7,8,9,10
4. The pass rate of the VENTANA HER2 Dual ISH DNA Probe Cocktail on HER2 Dual ISH 3-in-1 Xenograft Slides was determined by staining and analyzing 180 xenograft slides. Pass rate across all three platforms (BenchMark GX, BenchMark XT and BenchMark ULTRA instruments) was 92.8% with a two-sided 95% confidence interval of 88.0% to 95.7%.

TROUBLESHOOTING

1. If the xenograft slide is negative, it should be checked to ensure that the slide has the proper bar code label. Verify that the instrument, dispensers, and bulks are functioning as intended.
2. Refer to the probe method sheet Troubleshooting section for specific assay recommendations.

REFERENCES

1. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
2. Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.
3. Kraus MH, Popescu NC, Amsbaugh C, King CR. Overexpression of the EGF receptor-related proto-oncogene erbB-2 in human mammary tumor cell lines by different molecular mechanisms. EMBO J 1987;6:605-610.
4. Kury FD, Schneeberger C, Sliutz G, Kubista E, Salzer H, et al. Determination of HER2/neu amplification and expression in tumor tissue and cultured cells using a simple, phenol free method for nucleic acid isolation. Oncogene 1990;91403-1408.
5. Kallioniemi OP, Kallioniemi A, Kurisu W, Thor A, et al. ERBB2 amplification in breast cancer analyzed by fluorescence in situ hybridization. PNAS USA 1992;89:5321-5325.
6. Barlund M, Tirkkonene M, Forozan F, Tanner MM, Kallioniemi O, et al. Increased copy number at 17q22-q24 by CGH in breast cancer is due to high-level amplification of two separate regions. Genes Chrom Cancer 1997;20:372-376.
7. Revillion F, Hornez L, Peyrat JP. Quantification of c-erbB-2 gene expression in breast cancer by competitive RT-PCR. Clin Chem 1997;11:2114-2120.
8. Konecny G, Pauletti G, Pegram M, Untch M, Dandekar S, et al. Quantitative association between HER-2/neu and steroid hormone receptors in hormone receptor-positive primary breast cancer. JNCI 2003;95:142-153.
9. Nathanson DR, Nash GM, Chen B, Gerald W, Paty PB. Detection of HER-2/neu gene amplification in breast cancer using a novel polymerase chain reaction/ligase detection reaction technique. J Am Col Surg 2003;3:419-425.
10. Murthy SK, Magliocco AM, Demetrick DJ. Copy number analysis of c-erb-B2 (HER-2/neu) and Topoisomerase IIα genes in breast carcinoma by quantitative real time polymerase chain reaction using hybridization probes and fluorescence in situ hybridization. Arch Pathol Lab Med 2005;129:39-46.

NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

The summary of safety and performance can be found here:

https://ec.europa.eu/tools/eudamed

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

GTIN Global Trade Item Number

UDI Unique Device Identifier

INTELLECTUAL PROPERTY

CONTACT INFORMATION

Ventana Medical Systems, Inc.
1910 E. Innovation Park Drive
Tucson, Arizona 85755
USA
+1 520 887 2155
+1 800 227 2155 (USA)

www.roche.com

EC REP

Roche Diagnostics GmbH
Sandhofer Strasse 116
D-68305 Mannheim
Germany
+800 5505 6606

CE