Tissue-Tek Genie®

anti-HER2 Rabbit Monoclonal Antibody [EP3]

REF 8388-C010

Instructions for use

For *in vitro* diagnostic use.

Intended purpose

Intended use: The Tissue-Tek Genie® anti-HER2 Rabbit Monoclonal Antibody [EP3] is designed to qualitatively detect HER2 protein in formalin-fixed, paraffin-embedded (FFPE) human tissue specimen sections by immunohistochemistry (IHC) staining on the automated Tissue-Tek Genie® Advanced Staining System.

This device functions as an aid for diagnosis and shall be used by a qualified pathologist with a panel of other antibodies to characterize neoplasms with HER2 protein expression.

Limitations

The Tissue-Tek Genie® anti-HER2 Rabbit Monoclonal Antibody [EP3] has been optimized for use with the Tissue-Tek Genie® Advanced Staining System, Tissue-Tek Genie® reagents, and FFPE specimen sections. Staining quality may be diminished when used with other systems and/or reagents.

The clinical interpretation must be made in conjunction with histological examination, relevant clinical information, a panel of other antibodies, other diagnostic tests, and proper controls by a qualified pathologist.

Staining quality may be diminished by improper or incomplete removal of the paraffin.

The sensitivity of this antibody to identify the HER2 protein may be affected by improper specimen handling. This may alter antigenicity, weaken detection, and may generate false negative results.

Special processing of tissues such as decalcification of bone marrow tissues may lead to inconsistent staining.

Positively charged slides are recommended to obtain optimal staining with the Tissue-Tek Genie Advanced Staining System.

Summary and principle

Immunohistochemistry (IHC) staining is an established *in vitro* diagnostic method to visualize the presence of specific proteins expressed within a tissue section to study the microscopic features.

IHC staining is accomplished in two steps:

1) a primary antibody recognizes a particular target protein expressed on a specific cell compartment of a specific cell type on various tissues, and

2) a secondary and tertiary antibody conjugated to a chromogenic enzyme bind with the primary antibody for the detection of the antibody-antigen interaction. In chromogenic detection under a light microscope, an enzyme conjugated to the antibody cleaves a substrate to produce a colored precipitate at the location of the protein.

Human epidermal growth factor receptor 2 (HER2), also known as c-erbB2, ERBB2, and HER2/neu, is a transmembrane receptor tyrosine kinase.[1] It typically displays a predominantly membranous staining pattern,[2] with focal and rare membranous positivity in normal epithelial cells (up to moderate intensity in normal tonsillar and esophageal epithelium).[3,4] However, HER2 expression is generally absent in most normal tissue specimens[3,4] as well as lymphocytes.[5]

HER2 activation and overexpression cause malignant transformation. In human cancers, HER2 is activated via gene amplification on the long arm of chromosome 17.[6] HER2 is detected at various intensities in a range of neoplastic cells.[3,6-9] The analysis of HER2 expression in invasive breast carcinoma is standard in clinical practice and uses well-established scoring systems.[2,3,6-8] Amplification and overexpression of HER2 occur in approximately 10-25% of invasive breast carcinomas.[2,3,6-8,10] HER2-low expression (IHC 1+ and IHC 2+ without gene amplification) occurs in 31-71% of invasive breast carcinomas.[10,11] While HER2 expression in invasive breast carcinomas is frequently reported, recent studies have also highlighted the importance of HER2 in other neoplasms.[2,3,6-9] HER2 overexpression has been seen predominantly in malignancies of epithelial origin, such as carcinomas from the bladder, stomach, uterus, ovary, lung, and colon.[3,8,9] The variability of reported HER2

expression levels across different neoplasms is partly due to the lack of consensus on a standardized scoring system.[3,8,9] HER2 expression is absent in 100% of lymphomas.[5]

Anti-HER2 antibody labels HER2 in both normal and neoplastic cells and has a membranous staining pattern.

The Tissue-Tek Genie anti-HER2 Rabbit Monoclonal Antibody [EP3] is a primary antibody against the human HER2 protein and is provided in buffered saline containing 1% bovine serum albumin and 0.09% sodium azide. FFPE specimen sections are placed on positively charged slides and the paraffin is removed using the Tissue-Tek Genie® Dewax Solution (REF 8865-G001), after which heat-induced epitope retrieval is performed using the Tissue-Tek Genie® Citrate Antigen Retrieval Solution (REF 8742-G001).

IHC demonstration of HER2 protein in FFPE specimen sections is achieved through the use of the Tissue-Tek Genie anti-HER2 Rabbit Monoclonal Antibody [EP3] and the Tissue-Tek Genie® PRO Detection Kit, DAB (REF 8826-K250). This procedure entails the sequential application of antibody and kit components as follows:

- Tissue-Tek Genie® Protein Block
- Tissue-Tek Genie® anti-HER2 Rabbit Monoclonal Antibody [EP3]
- Tissue-Tek Genie® Peroxidase Block
- Tissue-Tek Genie® Link (binds to the primary antibody)
- Tissue-Tek Genie® Poly HRP-Conjugate (binds to the Link)
- Tissue-Tek Genie® DAB (visualizes the detected protein)
- Tissue-Tek Genie® DAB Intensifier

Tissue-Tek Genie® Hematoxylin (REF 8830-M250) is then used to visualize the nuclei of cells. The IHC stained slide is coverslipped and reviewed using a light microscope.

Expected results

Cellular staining pattern: membranous

Positive control tissue: breast carcinomas with weak, moderate and strong HER2 expression

The specificity and intended use of this antibody were validated by performing IHC staining on the Tissue-Tek Genie Advanced Staining System using normal and neoplastic FFPE tissue sections as follows.

Analytical sensitivity/specificity: A total of 32 types and 152 specimens of normal FFPE tissues were tested. Tissue-Tek Genie anti-HER2 Rabbit Monoclonal Antibody [EP3] exhibited weak membranous staining (<2+) in a small number of normal epithelial cells (11/134) of the skin, breast, esophagus, small intestine, colon/appendix, kidney, placenta, and cardiac muscle. Rare, focal membranous staining was observed in tonsillar epithelium (7/18). Staining was absent in most normal tissue specimens (123/152), including cerebrum (6/6), cerebellum (3/3), pituitary gland (1/1), thyroid gland (4/4), parathyroid gland (1/1), thymus gland (2/2), salivary gland (1/1), lung (7/7), stomach (6/6), pancreas (7/7), liver (8/8), spleen (7/7), adrenal gland (7/7), prostate gland (7/7), testis (7/7), cervix (3/3), bladder (3/3), fallopian tube (2/2), skeletal muscle (4/4), cardiac muscle (1/1), ovary (4/4), omentum (1/1), and peripheral nerve (1/1). No staining was also observed in lymphocytes in all tissues test (0/152).

Precision studies for Tissue-Tek Genie anti-HER2 Rabbit Monoclonal Antibody [EP3] lots will be completed. FFPE tissue sections of breast carcinoma will be used. Studies will be conducted to demonstrate reproducibility in lot-to-lot (minimum of 2 lots), run-to-run (minimum of 2 Genie runs), instrument-to-instrument (2 Genies), station-to-station (minimum of 2 stations), and operator-to-operator (2 operators). The results will be compared to their acceptance criteria: Weak to strong membranous staining in neoplastic cells of a subset of invasive breast carcinomas.

These results will demonstrate precision of the Tissue-Tek Genie anti-HER2 Rabbit Monoclonal Antibody [EP3], which was consistent across lots, runs, instruments, stations, and operators.

Diagnostic sensitivity/specificity: A total of 27 types and 517 specimens of neoplastic FFPE tissues were tested.

Various membranous staining intensities (0, 1+, 2+, and 3+) were detected in neoplastic cells of invasive breast carcinomas scored according to ASCO/CAP guidelines.[2]

Table 1. ASCO/CAP guidelines for HER2 IHC assessment in invasive breast carcinomas.

Score 0	No staining is observed, or incomplete membrane staining is observed in ≤10% of the tumor cells.
Score 1+	A faint perceptible and incomplete membrane staining is observed in > 10% of the tumor cells.
Score 2+	A weak to moderate circumferential incomplete membrane staining is observed in more than 10% of the tumor cells or an intense circumferential complete membranous staining in ≤10% of the tumor cells.
Score 3+	An intense circumferential complete membrane staining is observed in > 10% of the tumor cells.

In a study of invasive breast carcinoma tissue specimens (n=200) that represented the general population, Tissue-Tek Genie anti-HER2 Rabbit Monoclonal Antibody [EP3] demonstrated membranous staining of neoplastic cells in a subset of invasive ductal carcinomas (103/200, 51.5%). Of 200 breast carcinomas tested, 76 (38%) showed HER2-low expression, defined as 1+ or 2+ with unamplified status confirmed by FISH. In contrast, 27 carcinomas (13.5%) demonstrated HER2 overexpression, defined as 3+ or 2+ with amplified status confirmed by FISH.

Table 2. HER2 status in breast carcinoma from the general population

Category	HER2 Status	No. of Breast carcinoma	Percentage
Negative	0+	97	48.5%
Low expression	1+	67	38%
	2+ unamplified	9	

Overexpression	2+ amplified	5	13.5%
	3+	22	
	Total	200	100%

The antibody labeled (exhibited any positive membranous staining) 2/2 (100%) urothelial carcinomas, 1/3 (33%) gastric adenocarcinomas, and 2/5 (40%) colon adenocarcinomas. Other malignant neoplasms tested, including ones of lung, pancreas, thyroid, ovary, kidney, brain, skin (including melanoma), and smooth muscle origin were negative for HER2 (no staining observed).
Diagnostic specificity was demonstrated by the absence of staining in neoplastic cells of lymphomas (30/30, 100%), including non-Hodgkin B-cell lymphomas and T-cell lymphomas.

Clinical performance:

The Tissue-Tek Genie anti-HER2 Rabbit Monoclonal Antibody [EP3] demonstrated conformity with the expected clinical performance through analytical studies and assessments of diagnostic performance in conjunction with established scientific validity (summarized in the "Summary and principle" section above with data from the references at the end of this IFU) based on information on other IVD medical devices with the same antibody, textbooks, and available peer-reviewed literature.

Tissue specimen	Established validity	Tissue specimen tested
Normal epithelium, including tonsil	Focal and rare membranous HER2 positivity is observed in normal epithelial cells (up to moderate intensity in normal tonsillar and esophageal epithelium).[3,4]	Weak membranous staining (less than 2+) was observed in a small number of normal epithelial cells (11/134) of the skin, breast, esophagus, small intestine, colon/appendix, kidney, placenta, and cardiac muscle. Rare focal membranous staining was observed in the tonsillar epithelium (7/18).
Other normal tissue	HER2 expression is generally absent in most normal tissue specimens.[3,4]	No staining was observed in most normal tissue specimens (123/152), including cerebrum (6/6), cerebellum (3/3), pituitary gland (1/1), thyroid gland (4/4), parathyroid gland (1/1), thymus gland (2/2), salivary gland (1/1), lung (7/7), stomach (6/6), pancreas (7/7), liver (8/8), spleen (7/7), adrenal gland (7/7), prostate gland (7/7), testis (7/7), cervix (3/3), bladder (3/3), fallopian tube (2/2), skeletal muscle (4/4), cardiac muscle (1/1), ovary (4/4), omentum (1/1) and peripheral nerve (1/1).
Lymphocytes	HER2 expression is absent in lymphocytes.[5]	No staining was observed in lymphocytes in all tissues test (0/152).
Invasive breast carcinoma	Amplification and overexpression of HER2 occur in approximately 10-25% of invasive breast carcinomas.[2,3,6-8,10] HER2-low expression (IHC 1+ and IHC 2+ without gene amplification) occurs in 31-71% of all breast carcinomas.[10,11]	Of 200 breast carcinomas tested, 27 (13.5%) demonstrated HER2 overexpression, defined as 3+ or 2+ with amplified status confirmed by FISH. Of 200 breast carcinomas tested, 76 (38%) showed HER2-low expression, defined as 1+ or 2+ with unamplified status confirmed by FISH.
Lymphoma	HER2 expression is absent in 100% of lymphomas.[5]	HER2 was absent in neoplastic cells of lymphomas (30/30, 100%), including Non-Hodgkin B-cell lymphomas and T-cell lymphoma.

Together, this information is sufficient to demonstrate conformity with relevant essential principles without the need for additional clinical performance data.

Cautions and warnings

For professional use only.

Take reasonable precautions when handling. Avoid contact of reagents with eyes, skin, and mucous membranes. Wear protective gloves, protective clothing, and eye protection.

The product should not be exposed to temperatures outside of the storage conditions.

Capsules filled with ready-to-use, pre-diluted, antibody are for single use only. The in-use stability of each capsule is approximately 72

hours when used outside the storage conditions. Do not attempt to refill or add additional reagent. Discard capsule after use.

It is recommended to include appropriate controls on each specimen slide to help in identifying any deviation that might occur during the staining process.

All disposal practices must be in compliance with all Federal, State/Provincial and local laws and regulations. Refer to the SDS for further information.

Specimen collection and preparation for analysis

Routinely processed, formalin-fixed, paraffin-embedded tissues are suitable for use with this reagent when used with Tissue-Tek Genie reagents and a Tissue-Tek Genie Advanced Staining System (see section "Material required but not supplied"). The recommended fixation is performed using 10% (v/v) neutral buffered formalin for 24-72 hours.[12] Variable results may occur because of prolonged fixation or special processes such as decalcification of bone marrow preparations. Each cut section should be 3-5 µm in thickness and placed on a positively charged glass slide. Slides containing the tissue section may be baked for at least 30 minutes to overnight (typically up to 16 hours) in a 58-60°C oven.[12]

Storage conditions

Store this product at 2-8°C. Do not freeze. Return unused capsules to 2-8°C.

For the date of expiration, refer to the label on the product.

The reagent will be stable until its expiration date when stored and handled properly. Do not use the reagent beyond its assigned expiration date. Storage conditions other than those specified above must be verified by the user.

Do not use when precipitate is visible in the reagent container.

Instructions for use

Tissue-Tek Genie® anti-HER2 Rabbit Monoclonal Antibody [EP3], RTU, 10 capsules/pack (REF 8388-C010):

1. Place the Tissue-Tek Genie® Reagent Dispensing Area Tag (RDA-Tag) attached to the capsule into the RDA.
2. Push the capsule into the RDA with foil side down and click the attached RDA-Tag down into place on the RDA.
3. Place the RDA on the desired station of the Tissue-Tek Genie Advanced Staining System.
4. Place the slide with the specimen section on the same station, specimen section side down.
5. Assign protocol 8388 to the same station.
6. Initiate execution of protocol 8388.
7. The RDA-Tag 8388 will be scanned and registered automatically when the staining process is initiated.
8. During the primary antibody application step, the antibody will be released from the capsule into the RDA and onto the specimen section on the slide.
9. The staining protocol continues to the end.

Material required but not supplied

- Tissue-Tek Genie® Advanced Staining System (REF 8200)
- Positive and negative tissue controls
- Drying oven capable of maintaining a temperature of 58-60°C
- Tissue-Tek Genie® Dewax Solution (REF 8865-G001)
- Tissue-Tek Genie® Wash Buffer Solution (REF 8874-G004)
- Tissue-Tek Genie® Citrate Antigen Retrieval Solution (REF 8742-G001)
- Tissue-Tek Genie® Non-Immune Rabbit Ig Antibody, Negative Control (REF 8605-C010)
- Tissue-Tek Genie® PRO Detection Kit, DAB (REF 8826-K250)
- Tissue-Tek Genie® Hematoxylin (REF 8830-M250)
- Tissue-Tek Genie® Reagent Dispense Area [RDA] (REF 8616-G090)

Further information can be found on the Sakura Finetek USA website at **www.sakuraus.com/Genie**

Troubleshooting

Testing run should include proper reagent and tissue controls.

- If the positive control exhibits negative, or weaker, or stronger staining, or more background staining than expected, other positive controls on the same instrument run should be examined to determine if this is due to the antibody, other reagents, software, instrumentation, or the processing and fixation of tissue specimen(s).
- If the paraffin has not been removed completely, the deparaffinization procedure should be verified.
- If tissue sections have washed off, slides should be examined to ensure they are positively charged, and the specimen should be examined for possible inadequate processing or fixation.
- Refer to the Tissue-Tek Genie Advanced Staining System operating manual or contact your Sakura Finetek Technical support representative for information or assistance.

Order information / product provided

Product code, product name and quantity

REF 8388-C010 Tissue-Tek Genie® anti-HER2 Rabbit Monoclonal Antibody [EP3]; RTU, 10 capsules/pack.

NOTE: Not available in the U.S.A.

NOTE: The Safety Data Sheet (SDS) is available online on the Sakura Finetek USA website at **www.sakuraus.com/SDS.html**

The Summary of Safety and Performance (SSP) is available online via EUDAMED.

References

1. King CR, Kraus MH, Aaronson SA. Amplification of a novel v-erbB-related gene in a human mammary carcinoma. Science. 1985; 229(4717): 974-6. https://doi.org/10.1126/science.2992089.
2. Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, Allred DC, Bartlett JM, Bilous M, Fitzgibbons P, Hanna W, Jenkins RB, Mangu PB, Paik S, Perez EA, Press MF, Spears PA, Vance GH, Viale G, Hayes DF, American Society of Clinical O, College of American P. 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. 2013; 31(31): 3997-4013. https://doi.org/10.1200/JCO.2013.50.9984.
3. Natali PG, Nicotra MR, Bigotti A, Venturo I, Slamon DJ, Fendly BM, Ullrich A. Expression of the p185 encoded by HER2 oncogene in normal and transformed human tissues. Int J Cancer. 1990; 45(3): 457-61. https://doi.org/10.1002/ijc.2910450314.
4. Press MF, Cordon-Cardo C, Slamon DJ. Expression of the HER-2/neu proto-oncogene in normal human adult and fetal tissues. Oncogene. 1990; 5(7): 953-62.
5. Bairey O, Pazgal I, Okon E, Shaklai M, Morgenshtern S. Lack of HER-2/neu expression in Hodgkin and non-Hodgkin lymphoma. Arch Pathol Lab Med. 2002; 126(5): 574-6. https://doi.org/10.5858/2002-126-0574-LOHNEI.
6. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science. 1987; 235(4785): 177-82. https://doi.org/10.1126/science.3798106.
7. Lebeau A, Deimling D, Kaltz C, Sendelhofert A, Iff A, Luthardt B, Untch M, Lohrs U. Her-2/neu analysis in archival tissue samples of human breast cancer: comparison of immunohistochemistry and fluorescence in situ hybridization. J Clin Oncol. 2001; 19(2): 354-63. https://doi.org/10.1200/JCO.2001.19.2.354.
8. Yan M, Schwaederle M, Arguello D, Millis SZ, Gatalica Z, Kurzrock R. HER2 expression status in diverse cancers: review of results from 37,992 patients. Cancer Metastasis Rev. 2015; 34(1): 157-64. https://doi.org/10.1007/s10555-015-9552-6.
9. Tapia C, Glatz K, Novotny H, Lugli A, Horcic M, Seemayer CA, Tornillo L, Terracciano L, Spichtin H, Mirlacher M, Simon R, Sauter G. Close association between HER-2 amplification and overexpression in human tumors of non-breast origin. Mod Pathol. 2007; 20(2): 192-8. https://doi.org/10.1038/modpathol.3800729.
10. Zhang H, Katerji H, Turner BM, Audeh W, Hicks DG. HER2-low breast cancers: incidence, HER2 staining patterns, clinicopathologic features, MammaPrint and BluePrint genomic profiles. Mod Pathol. 2022; 35(8): 1075-1082. https://doi.org/10.1038/s41379-022-01019-5.
11. Nishimura R, Fujiki Y, Taira T, Miyaki T, Kanemitsu S, Yotsumoto D, Teraoka M, Kawano J, Gondo N, Mitsueda R, Baba S, Ohi Y, Rai Y, Sagara Y, Sagara Y. The Clinicopathological and Prognostic Significance of HER2-Low Breast Cancer: A Comparative Analysis Between HER2-Low and HER2-Zero Subtypes. Clin Breast Cancer. 2024; 24(5): 431-438. https://doi.org/10.1016/j.clbc.2024.02.013.
12. Lott R, Tunnicliffe J, Sheppard E, Santiago J, Hladik C, Nasim M, Zeitner K, Haas T, Kohl S, Movahedi-Lankarani S. Practical Guide to Specimen Handling in Surgical Pathology. 2022. College of American Pathologists (CAP) and National Society for Histotechnology (NSH): Northfield, IL.

Contact

If located within the United States, contact Sakura Finetek USA, Inc. by calling toll free **1-800-725-8723** or contact your Sakura Finetek representative or authorized distributor.

In countries other than the United States, contact the nearest authorized Sakura Finetek instrument distributor or representative. Contact details may be found at **www.sakura.com**

Any incident should be reported to the manufacturer. In the European Union, any serious incident that has occurred in relation to the device shall be reported to the manufacturer, authorized representative, and the competent authority of the appropriate Member State in which the user and/or the patient is established.

Symbols

REF	Catalog number
LOT	Batch code
IVD	*in vitro* diagnostic medical device
	Temperature limitation
	Use by
	Manufacturer
	Consult instructions for use
CE	European Conformity
EC REP	Authorized representative in the European Community
	Do not re-use (applies to capsule format only)

LOT Please see product label for lot and expiration date information and if available the date of manufacture

Storage: 2°C 8°C

IVD

CE
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