Estrogen Receptor (SP1)
Catalog # RM-9101-S0, -S1, or -S (0.1ml, 0.5ml, or 1.0ml)
Catalog # RM-9101-R7 (7.0ml)

FOR IN VITRO DIAGNOSTIC USE

INDICATIONS AND USE

Intended Use

NeoMarkers Rabbit Monoclonal Anti-Human Estrogen Receptor Antibody (Clone SP1) is an immunohistochemical (IHC) assay intended for laboratory use for the qualitative detection of estrogen receptor (ER) antigen by light microscopy in sections of formalin fixed, paraffin embedded normal and neoplastic tissues on a Lab Vision automated slide stainer. It is indicated as an aid in assessing the likelihood of response to therapy as well as in the prognosis and management of breast cancer patients.

Summary and Explanation

Immunohistochemistry has been used to detect specific antigens in cells or tissue since 1950. The use of enzymes and peroxidase as markers for immunohistochemistry was reported by Nakane and Pierce in 1967. The increased sensitivity of the avidin-biotin-peroxidase detection system over the enzyme labeled antibody method was documented by Hsu et al. in 1981.

Determination of ER status for all primary breast carcinomas was recommended by the NIH in 1979, in order to better determine appropriate therapy. In 1985 both the NIH and the American Cancer Society independently published reports in support of determining hormone receptor status as an aid in the management of breast cancer. A number of methodologies to assess ER status have been in use. FDA – cleared therapies include cytosol receptor assay (SBA/DCC) analyzed by Scatchard plot (1981), histochemical analysis of tissue using fluorescent microscopy, histochemical analysis of frozen tissue using anti-ER rat monoclonal antibody conjugate (1988), and enzyme immuno-assay (EIA) also using anti-ER rat monoclonal antibody conjugate (1988). The immunohistochemical detection of ER has been described in cultured human breast cells, some human breast cancer tissues, human endometrium, some endometrial cancers, some low-grade endometrial stromal sarcomas, some cultured endometrial cells, some sweat gland tumors, some benign thyroid disease tissues, some thyroid cancers, some gastric cancers, some prostatic carcinomas and some female human bladders.

Principle of Procedure

Lab Vision’s NeMarkers rabbit monoclonal anti-ER antibody (Clone SP1) binds to ER in the paraffin embedded tissue section. The specific antibody is localized by a biotin conjugated secondary antibody formulation that recognizes rabbit immunoglobulins. This step is followed by the addition of an avidin/streptavidin enzyme conjugate that binds to the biotin present on the secondary antibody. The specific antibody secondary antibody avidin/streptavidin enzyme complex is then visualized with a precipitating enzyme reaction product, which is readily detected by light microscopy. Each step is incubated for a precise time and at room temperature. At the end of each incubation step, the Lab Vision automated slide stainer (Lab Vision Autostainer) washes the sections to stop the reaction and remove unbound material that would interfere the desired reaction in subsequent steps.

Clinical cases should be evaluated within the context of the performance of appropriate controls. Lab Vision recommends the inclusion of a positive tissue control fixed and processed in the same manner as the patient specimen placed on every slide run in addition to the case tissue. In addition to staining with NeoMarkers rabbit monoclonal anti-ER antibody (Clone SP1), a second slide should be stained with Negative Control for Rabbit IgG. For the test to be considered valid, the positive control tissue should exhibit nuclear staining of the tumor cells, normal breast glands or endometrial glands and stroma. These components should be negative when stained with Negative Control for Rabbit IgG. In addition, it is recommended that a negative tissue control slide be included for every batch of samples processed and run on Lab Vision Autostainer. This negative tissue control should be stained with NeoMarkers rabbit monoclonal anti-ER antibody (Clone SP1) to ensure that the antigen enhancement and other treatment procedures did not create false positive staining.

MATERIALS AND METHODS

Reagents

1. #RM-9101-S, (or -S0, -S1) is 1ml (or 0.1ml, 0.5ml) concentrated tissue culture supernatant containing rabbit anti-human monoclonal antibody directed against ER antigen, with 0.05% Sodium Azide.

2. #RM-9101-R7 (Ready to Use) is provided as a rabbit anti-human monoclonal antibody prediluted in 0.05mol/L Tris-HCl, pH 7.6 containing stabilizing protein and 0.015mol/L sodium azide.

A peptide representing the C-terminal of human ER alpha protein was synthesized and covalently conjugated with keyhole limpet haemocyanin (KLH). New Zealand White rabbits were immunized. The sera were tested by immunoassay and immunohistochemical staining. The rabbit with the best titer in the immunoassay and IHC was selected for a final intravenous boost four days before removal of the spleen. Fusions were performed using lymphocytes from an immobilized rabbit and the fusion partner. The hybridomas were sub-cloned by limit dilution. The final antibody is produced from hybridoma culture supernatants using serum free media with no further purification. The antibody is diluted in 10mM phosphate buffered saline with 0.3% carrier protein and 0.05% sodium azide. The total protein concentration of the reagent is approximately 0.4 mg/ml. Specific antibody concentration is approximately 160µg/ml in the concentrate format and 1.6µg/ml in the ready-to-use format. There are no known irrelevant antibodies in the preparation.

Reconstitution, Mixing, Dilution, Titration

NeoMarkers Ready-To-Use antibodies (#RM-9101-R7) have been optimized for use on a Lab Vision Autostainer in combination with Lab Vision UltraVision Detection Systems (see Instructions for Use), and should not require further dilution. No reconstitution or mixing is required. Further dilution may result in loss of sensitivity. The user must validate any such change.

NeoMarkers concentrated antibodies (#RM-9101-S, -S1, -S0) must be diluted in accordance with the staining procedure when used with Lab Vision UltraVision Detection Systems (see Instructions for Use recommended dilution). Use of non-Lab Vision systems other than recommended systems and protocols require validation by the user.
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Differences in tissue processing and technical procedures in the laboratory may produce significant variability in results and require regular use of controls, see Quality Control Procedure section.

Materials and Reagents Needed But Not Provided

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

1. NeoMarkers Negative Control for Rabbit IgG  
2. Lab Vision Antibody Diluent (Catalog # TA-125-UD)  
3. Lab Vision Autostainer Systems  
4. Microtome  
5. Microscope slides, treated to enhance tissue adherence  
6. Positive and negative tissue controls  
7. Bar code labels (appropriate for negative control and primary antibody being tested)  
8. Drying oven capable of maintaining a temperature of 70°C +/- 5°C  
9. 10% neutral buffered formalin  
10. Timer  
11. Staining Dishes  
12. Ethanol or reagent alcohol  
13. Countertop mounted tissue pretreatment reagents  
14. Deionized or distilled water  
15. Microscope slides, treated to enhance tissue adherence  
16. Epitope recovery/tissue pretreatment reagents  
17. Lab Vision UltraVision Immunohistochemistry Detection Systems  
18. Lab Vision Autostainer Systems  
19. Chromogens  
20. Counter stains and mounting media  
21. Light microscope

Storage and Handling

Store NeoMarkers rabbit monoclonal anti-ER antibody (Clone SP1) at 2-8°C. This product contains sodium azide and is stable for 24 months when stored at 2-8°C. Do not use after expiration date indicated on label of the product. If reagent is not stored as recommended, performance must be validated by the user. Lab Vision’s NeoMarkers rabbit monoclonal anti-ER antibody (Clone SP1) can be used immediately after removal from storage.

Indications of Instability

When properly stored, the reagent should be stable to the dating indicated on the label. NeoMarkers rabbit monoclonal anti-ER (Clone SP1) has designed the ER primary antibody (Clone SP1) to have a 24 month stability from the date of manufacture. The user must honor the expiration date on the label. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with unknown specimens. Positive controls ensure that the specimen staining was carried out correctly. Negative controls are used to assess non-specific staining, which must be taken into consideration when interpreting results. Whenever positive control material shows a decrease in staining, it is a possible indication of reagent instability and Lab Vision customer Service (1-510-991-2800 / 1-800-828-1628) should be contacted immediately.

Specimen Collection and Preparation for Analysis

Formalin-fixed, paraffin-embedded tissues are suitable for use with Lab Vision’s NeoMarkers rabbit monoclonal anti-ER antibody (Clone SP1) when used with Lab Vision’s UltraVision Detection Systems and Lab Vision Autostainer.

For Formalin-fixed, Paraffin-Embedded Tissues:

Tissue specimens should be preserved by 10% buffered formalin (pH 7.4) as the tissue fixative followed by paraffin embedding. Paraffin-embedded tissue sections should be cut at 3-5 micrometer thick and mounted on treated glass slides to enhance tissue adherence. Tissue slides should be dried at 60-70°C for 1-2 hours. Alternatively, tissue slides can be dried overnight at 37°C. Cool to room temperature if slides are to be stored.

Manual Deparaffinization Procedure:

1. De-wax slides by immersing in xylene for 3 x 5 minutes.
2. Hydrate slides in 100%, 100%, 95 %, 80% ethanol for 3 minutes each, then immerse slides in tap water for 5 minutes. Do not allow slides to dry.

Endogenous Peroxidase Quenching (for horseradish peroxidase detection method):

Immerse slides in 3% hydrogen peroxide solution for 10 minutes, then wash slides in PBS for 2 x 3 minutes. Do not allow slides to dry.

Pretreatment/Antigen Retrieval:

Heat Induced Epitope Retrieval (HIER) is recommended:

a. Place slides in Citrate Buffer, pH 6.0 (Catalog # AP-9003-500).

b. Heat slides to near boiling (95°C-98°C) for 10-20 minutes. Some may require longer heating times and/or higher temperatures. Cool slides in buffer at room temperature for at least 20 minutes before proceeding.

c. Rinse in PBS at least 3 x 1 minute before proceeding. Do not allow slides to dry.

INSTRUCTIONS FOR USE

NeoMarkers primary antibodies have been developed for use on a Lab Vision Autostainer in combination with Lab Vision UltraVision Detection Systems and accessories. The recommended procedures for NeoMarkers Anti-ER antibody (Clone SP1) are as follows:

- Tissue Section Pretreatment: Citrate Buffer, pH 6.0, 10 minutes at 95°C-98°C, cool 20 minutes at room temperature.
- Apply primary antibody:
  - Dilution of Concentrated Antibody (#RM-9101-S, -S1, -S0): 1:100 to 1:400 in antibody diluent (we recommend users to apply the diluted antibody to the slide as soon as possible)
  - Or Ready-To-Use Antibody (#RM-9101-R7): No further dilution, reconstitution, or mixing is required.
- Primary Antibody Incubation Time: 30 minutes at room temperature.
- Visualization: To detect antibody, follow the instructions provided with the visualization system. The following are instructions specific to Lab Vision’s UltraVision Detection Kit #TP-015-HA:
  - Cover tissue with 4-5 drops of UltraVision biotinylated goat anti-polyvalent secondary antibody, incubate at room temperature for 10 minutes, then wash in PBS for 3 x 3 minutes.
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- Add drops of streptavidin peroxidase to cover section, incubate at room temperature for 10 minutes, then wash in PBS for 3 x 3 minutes.
- Add 20-50µl (1 drop) of AEC Chromogen to 1 ml of AEC Substrate and mix well. Apply mixture to tissue section within 20 minutes of mixing. Incubate tissue section for 5-20 minutes, depending on the desired stain intensity.
  - • Counterstain with hematoxylin according to your laboratory’s procedures.
  - • Positive Control Tissue: Breast carcinoma known to be positive for estrogen receptor.

Using Lab Vision Autostainer:
- • Apply slide barcode label, which corresponds to antibody protocol to be performed. Or, program Lab Vision Autostainer for the protocol you wish to use (See Autostainer instruction manual).
- • Load slides and scan barcodes. Lab Vision Autostainer will indicate which reagents are necessary.
- • Load reagents as indicated by Lab Vision Autostainer reagent map.
- • Start the staining run.
- • At the completion of the run, remove slides from the automated slide stainer.
- • Counterstain if necessary.
- • For DAB, dehydrate, clear and Coverslip with permanent mounting media.
- • For AEC or Fast Red, do not dehydrate and clear. Mount with aqueous mounting medium.
- • The stained slides should be read within two to three days of staining and are stable for at least two years if properly stored at room temperature (15 to 25°C).

SAFETY ISSUES
Precautions
1. This antibody is intended for in vitro diagnostic use.
2. Take reasonable precautions when handling reagents. Use disposable gloves when handling suspected carcinogens or toxic materials, for example xylene or formaldehyde.
3. Avoid contact of eyes, skin and mucous membranes with reagents. If reagent comes in contact with sensitive areas, wash with copious amounts of water.
4. Patient specimens and all materials should be handled as if capable of transmitting infection and disposed of with proper precautions.
5. Never pipette by mouth.
6. Avoid microbial contamination of reagents as this could produce incorrect results.
7. Incubation times or temperatures other than those specified may give erroneous results. Any such change must be validated by the user.
8. The reagents have been optimally diluted and further dilution may result in loss of antigen staining. Any such change must be validated.

Warnings
The reagents contain sodium azide. Avoid contact with skin and mucous membranes. Flush affected areas with copious amounts of water. Seek immediate medical attention if reagents are splashed in eye or ingested.

Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up. Clean exposed metal surfaces with 10% sodium hydroxide.

QUALITY CONTROL PROCEDURE
Positive Tissue Control
A positive tissue control must be run with every staining procedure performed. The positive staining cells or tissue components (nuclear staining of tumor cells and normal gland cells) are used to confirm that the antibody was applied and the instrument functioned properly. This tissue may contain both positive and negative staining cells or tissue components and serve as both the positive and negative control tissue. Control tissues should be fresh autopsy, biopsy or surgical specimens prepared or fixed as soon as possible in a manner identical to the test sections. Such tissues may monitor all steps of the procedure, from tissue preparation through staining. Use of a tissue sample fixed or processed differently from the test specimen will provide control for all reagents and method steps except fixation and tissue processing. This positive tissue control should only 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 positive staining, results with the test specimens should be considered invalid.

Negative Tissue Control
Use a tissue control known to be negative for estrogen receptor and to be fixed, processed and embedded in a manner identical to the patient sample(s) with each staining run to verify the specificity of NeoMarkers anti-ER antibody (Clone SP1) for demonstration of ER, and to provide an indication of specific background staining (false positive staining). Also a variety of different cell types in most tissue sections can be used by the laboratorian as internal negative control to verify NeoMarkers anti-ER antibody (Clone SP1) performance specifications. There should be no positive staining of the negative tissue control. If specific staining occurs in the negative tissue control sites, results with the patient specimens should be considered invalid.

Negative Reagent Control
A negative reagent control should be run for every specimen to aid in the interpretation of results. A negative reagent control is one that is not specific for estrogen receptor and is used in place of the primary antibody to check nonspecific staining. This provides an indication of nonspecific background staining for each slide. Any staining observed on the specimen is probably due to non-specific protein binding or non-specific binding of other reagents.

Assay Verification
Prior to initial use of this antibody in the user’s laboratory or if there is a change of lot number, the specificity of the antibody should be verified by staining a number of positive and negative tissues with known performance characteristics. These quality control tests should be repeated for each new lot or whenever there is a change of a lot number or a change in assay parameters. Refer to the quality control procedure previously outlined in this section of the product insert and to the quality control recommendations of the CAP certification program for immunohistochemistry as well as the NCCCLS IHC guideline.
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Assay verification on a daily basis may be accomplished through the proper use of the above-mentioned positive and negative controls. In addition, it is recommended that on a monthly basis, the ER positive tissue control be stained and compared to the same tissue control stained in previous month. Comparison of controls stained at monthly intervals serves to monitor the assay stability, sensitivity, specificity, and reproducibility. All quality control requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Interpretation of Results
The Lab Vision automated immunostaining procedure results in a colored reaction product to precipitate at the antigen sites localized by NeoMarkers anti-ER antibody (Clone SP1). The cellular staining pattern for NeoMarkers anti-ER antibody (Clone SP1) is nuclear. Cytoplasmic staining is considered to be background, non-specific staining, if seen. Nuclei of positive tissue control should be positively stained, whereas nuclei of negative tissue control should not be stained. If the positive tissue control fails to give appropriate positive staining, or if specific staining is found in the negative tissue control, results with the test specimens should be considered invalid. Lab Vision recommends using a semi-quantitative scoring system for staining intensity, e.g., 0-3 for negative, weak, moderate and strong staining.

LIMITATIONS

General Limitations
1. Immunohistochemistry (IHC) is a multistep diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selection, fixation, processing, preparation of the IHC slide, and interpretation of the staining results. Excessive or incomplete counterstaining may compromise proper interpretation of results.
2. Tissue 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, antibody trapping, or false negative results. Inconsistent results may be due to variations in fixation and embedding methods, or to inherent irregularities with the tissue.
3. Excessive or incomplete counterstaining may compromise proper interpretation of results.
4. The clinical interpretation of any positive staining, or its absence must be evaluated within the context of clinical history, morphology and other histopathological criteria. The clinical interpretation of any staining, or its absence must be complemented by morphological studies and proper controls as well as other diagnostic tests. This antibody is intended to be used in a panel of antibodies. It is the responsibility of a qualified pathologist to be familiar with the antibodies, reagents and methods used to product the stained preparation. Staining must be performed in a certified licensed laboratory under the supervision of a pathologist responsible for reviewing the stained slides and assuring the adequacy of the positive and negative controls.
5. Lab Vision provides antibodies and reagents at optimal dilution for use when the provided instructions are followed. Any variation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results.
6. The product is not intended for use in flow cytometry, performance characteristics have not been determined.
7. 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 variability of antigen expression in neoplasms, or other pathological tissues.
Contact Lab Vision with documented unexpected reactions.
8. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HbsAg) may exhibit nonspecific staining with horseradish peroxidase.
9. When used in blocking steps, normal sera from the same animal source as the secondary antiserum may cause false negative or false positive results because of autoantibodies or natural antibodies.
10. False positive results may be seen due to non immunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (e.g. erythrocytes), or endogenous peroxidase activity (Cytochrome C) or endogenous biotin (liver, breast, brain kidney) depending on the type of immunostain used.
11. As with any immunohistochemical test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells/tissues assayed.

Specific Limitations
1. Lab Vision’s NeoMarkers rabbit monoclonal anti-ER antibody (Clone SP1) has been optimized for a 30 minute incubation time, with antigen enhanced tissue. Due to variation in tissue processing, however, it may be necessary to increase or decrease the ER Primary Antibody incubation time on individual specimens. Users who deviate from the recommended test procedures must accept responsibility for interpretation of patient results under these circumstances.
2. Primary antibody incubation time depends on the degree of tissue fixation and may range from 4 to 32 minutes. Lab Vision recommends 30 minutes for use with its detection kits. For further information on fixation variables refer to Immunomicroscopy: A Diagnostice Tool for the Surgical Pathologist.
3. The following normal tissue was not tested: parathyroid. The user should determine appropriate staining in the above tissue prior to interpretation of staining information.
4. The ER Primary Antibody (clone SP1) negative result does not exclude the presence of ER. Negative reactions in breast carcinomas may be due to loss or marked decrease of expression of antigen. Therefore, it is recommended that this antibody be used in a panel of antibodies including progesterone receptor.

SUMMARY OF EXPECTED RESULTS
Immunoreactivity of NeoMarkers rabbit monoclonal anti-ER antibody (Clone SP1) was demonstrated by a study using clinical specimens that showed appropriate staining in formalin fixed, paraffin embedded breast carcinoma tissue.
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**Performance Characteristics:**
A Positive staining result is defined as more than 10% of tumor cells with stained nuclei of any intensity.

**Method Comparison:**
When compared to a predicate device, NeoMarkers rabbit monoclonal anti-ER antibody (Clone SP1) showed agreement of 90.76% (226/249), with a 95% confidence interval of 86.46-94.05%. Positive percent agreement is 91.07% (102/112), with a 95% confidence interval of 84.19-95.64%. Negative percent agreement is 90.51% (124/137), with a 95% confidence interval of 84.32-94.85%.

In addition, Cano et al. investigated the ER status on 40 alcohol-fixed smears by fine-needle aspiration, and paraffin sections from breast cancer patients (39 women and 1 man, ages 41–82 yr) using NeoMarkers’ rabbit monoclonal antibody anti-ER (Clone SP1) without antigen retrieval. The results were compared with assessment by the classic method (with antigen retrieval) with a mouse monoclonal anti-ER antibody (Clone 6F11). ER detection in paraffin sections using antigen retrieval SP1 had a sensitivity of 100% (24/24), a specificity of 100% (16/16) and an accuracy of 100% (40/40) when compared to antibody 6F11.

**Specificity:**
Specificity of NeoMarkers rabbit monoclonal anti-ER antibody (Clone SP1) was determined by a study that tested formalin fixed, paraffin embedded normal tissues. The 30 normal tissues examined included adrenal, bone marrow, breast, cerebrum, cervix, colon, endometrium, esophagus, heart, kidney, liver, lung, mesothelium, ovary, pancreas, pituitary, testis, thyroid, prostate, stomach, small intestine, salivary gland, skeletal muscle, skin, placenta, cerebellum, spleen, tonsil, thymus, peripheral nerve. NeoMarkers rabbit monoclonal anti-ER antibody (Clone SP1) showed specific staining of nucleus in normal breast, uterus, and cervix tissues. This is expected, as it is known estrogen receptors are present in these tissues. Lab Vision also tested 40 neoplastic tissues using the same method as for normal tissue testing. The tissues examined included lung, esophagus, stomach, small intestine, colon, rectum, liver, pancreas, kidney, prostate, thyroid, smooth muscle, soft tissue, bone, leg, lip, skin, nerve, bronch, lymphatic system, testis, placenta, bladder, ovary, uterus, and breast. All cases tested negative for ER with the exception of ovary, ovary and breast. NeoMarkers rabbit monoclonal anti-ER antibody (Clone SP1) showed no specific positive staining in tumor tissue that is not expected to have ER. Sensitivity is dependent upon the preservation of the antigen. Any improper tissue handling during fixation, sectioning, embedding or storage, which alters antigenicity, weakens ER detection by NeoMarkers rabbit monoclonal anti-ER antibody (Clone SP1).

**Reproducibility:**
Reproducibility of staining with NeoMarkers rabbit monoclonal anti-ER antibody (Clone SP1) was determined by staining 10 slides containing the same tissue for Intra-run and 10 slides each day for ten days for Inter-run reproducibility. Results between slides showed no variation in staining intensity. Users should verify within run reproducibility results by staining several sets of serial sections with low, medium, and high antigen density in a single run (Intra-run) or on different days (Inter-run).

**REFERENCES**


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29. ODE Guidance documents, Center for Devices and Radiological Health. Draft: Premarketing approval review criteria for premarket approval of estrogen (ER) or progesterone (PGR) receptors in vitro diagnostic devices using steroid hormone binding (SBA) with dextran coated-charcoal (DCC) separation, histochemical receptor binding assays, or solid phase enzyme immunoassay (EIA) methodologies. Updated 7-29-1996.


Manufacturer
NeoMarkers, Inc.
46360 Fremont Blvd.
Fremont, CA 94538
Telephone: (510) 771-1560
Facsimile: (510) 771-1570
Toll Free: (800) 828 1628
Web Site: http://www.neomarkers.com

Other Countries:
Please contact your local Representative

Authorized Representative in U.K.:
Thermo Fisher Scientific
Anatomical Pathology
93-96 Chadwick Road, Astmoor
Runcorn, Cheshire WA7 1PR, UK
Tel: 44-1928-562600
Fax: 44-1928-562627

Revision: 090309C

@thermofisher.com

Thermo Fisher Scientific
Anatomical Pathology
48360 Fremont Blvd.
Fremont, CA 94538, USA
Tel: 1-510-771-1560
Fax: 1-510-771-1570
http://www.thermo.com/labvision

Labvision.uk
@thermofisher.co
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