

Kappa Probe PR214-100E

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Recommended detection system:

One Step polymer HRP ISH Detection System

Intended Use

BioGenex KAPPA PROBE is currently available for in vitro diagnostic use. This probe is designed for the specific localization of Kappa light chain mRNA in formalin-fixed, paraffin-embedded human tissues and/or frozen tissues by in situ hybridization.

Summary and Explanation

The oligonucleotide probe detects Kappa light chain mRNA in formalin-fixed, paraffin-embedded human tissues by in situ hybridization. The light chains of immunoglobulin molecules have two antigenic types: kappa and lambda. A given immunoglobulin molecule contains two identical light chains, either kappa or lambda. Therefore, the clonal nature of any immunoglobulin producing cell population can be determined by the light chain structure of the immunoglobulin that the cell produces. Kappa light chain restriction and its mRNA levels can be determined using in situ hybridization techniques. A mixture of non specific oligonucleotides that are of similar length to that of the Kappa probe is provided as the negative control.

Principles of the Procedure

In situ hybridization (ISH) allows the detection and localization of definitive nucleic acid sequences directly within a cell or tissue. High specificity is ensured through the action of annealing of probe nucleic acid sequence to complementary target nucleic acid sequence. ISH techniques can be used to identify infectious agents in tissue sections, to localize gene expression within individual cells, or to detect specific DNA sequences in the genome of cells.

In ISH, fixed tissue sections are deproteinized to expose target DNA or mRNA sequences. A hapten (fluorescein or digoxigenin-labeled probe) is hybridized to the exposed target DNA or mRNA sequences in the cells. Subsequent washing steps remove any probe that is not bound or that is non-specifically bound to the tissue section. An immunohistochemical (IHC) procedure is then used to detect the probe-target hybrid. (Down stream detection of hybridized hapten labeled probe is done by using specific anti-hapten antibody). This procedure includes incubating the slide with a mouse anti- fluorescein or digoxigenin antibody, followed by detection of this antibody with a second antibody enzyme conjugate. After addition of an appropriate substrate for the enzyme (such as DAB,-diaminobenzidine-solution), a colored reaction product is precipitated at the location of the probe-target hybrid. Microscopic examination of the slide provides visual interpretation of the staining results.

Reagents Provided

1 x 0.650 ml of fluoresceinated oligonucleotide Kappa probe in hybridization solution.

Materials Required But Not Provided

All the reagents and materials required for in situ hybridization are not provided. Pre-treatment reagents, super sensitive detection systems, control slides, control reagents and other ancillary reagents are available from BioGenex. Please refer to the product insert(s) of the BioGenex Super Sensitive One Step Polymer HRP ISH detection systems for detailed protocols and instructions.

Storage and Handling

Store the probe at 2-8°C. Warm to room temperature immediately prior to use (HPV probes may need water baths higher than 37° C to dissolve the precipitate in the probe).

This probe is suitable for use till expiry date when stored at 2-8°C. Do not use the product after expiration date printed on vial. If reagents are stored under any conditions other than those specified in the package insert, they must be verified by the user.

Positive and negative controls should be run simultaneously for every experiment. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact BioGenex Technical Support at **1-800-421-4149** or your local distributor.

Specimen Collection and Preparation

Tissues fixed in 10% (v/v) formalin are suitable for use prior to paraffin embedding. Consult references (Kiernan, 1981 ; Sheehan & Hrapchak, 1980) for further details on specimen preparation. Over-fixation may require prolonged incubation with Proteinase K and result in weak staining of positive tissue. Tissue processing conditions should be standardized in order to obtain consistent, reliable results. Frozen sections do not need proteinase K digestion.

Treatment of Tissues Prior to Staining

All formalin-fixed, paraffin-embedded tissue sections require pretreatment with Proteinase K following the instruction product data sheet.

Precautions

When the target to be detected is RNA, it is important to avoid contamination of the slides and reagents by ribonucleases (RNases—enzymes that degrade RNA) prior to and during hybridization. Be sure to wear gloves up to the hybridization step. All the reagents to be used up to the hybridization step are provided as RNase-free. Reagents to be prepared prior to use by users should also be prepared under RNase-free conditions.



Rep 2;R61 = May cause harm to the unborn child.

S38 S39 S45 S53 S60 P11 = In case of insufficient ventilation, wear suitable respiratory equipment. Wear eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Avoid exposure – obtain special instructions before use. This material and its container must be disposed of as hazardous waste.

For more information, refer to the Material Safety Data Sheet.

Staining procedure

- The BioGenex KAPPA PROBE is used without further dilution.
- The probe solution is brought to room temperature just prior to use.
- Formalin-fixed, paraffin-embedded tissue sections need pretreatment with Proteinase K.
- The testing parameters and testing protocols are listed in Table 1 below.
- The BioGenex Super Sensitive™ ONE STEP POLYMER ISH Detection System (DF400-50KE) is recommended for the staining.
- After staining, the slides are dehydrated in 100% reagent alcohol and cleared in xylene.
- Permanent mounting medium is applied to the slides.
- The negative control probe is run in parallel with the KAPPA PROBE

Reagent	Time / Temperature
Proteinase K	20 minutes at 37° C
Probe	10 minutes at 92° C followed by 60 minutes at 37° C
Wash Solution A	5 minutes at 45° C
Wash Solution B	5 minutes at 55° C
Polymer HRP	30 minutes at Room Temperature

- To prepare DAB -Add two drops or ~ 80ul of liquid DAB chromogen to 1 ml ready-to-use Substrate buffer before use

TESTING PARAMETERS

Dispensing pattern : 1/3

Dispensing volume: 25 µl

Quality Control

The recommended positive control tissue for this probe is Small Intestine. Refer to the appropriate detection system package insert for guidance on general quality control procedures.

Troubleshooting

Refer to the troubleshooting section in the package inserts of BioGenex Super Sensitive Detection Systems (or other equivalent detection systems) for remedial actions on detection system related issues, or contact BioGenex Technical Service Department at **1-800-421-4149** or your local distributor to report unusual staining.

Expected Results

Proper use of this probe and Super Sensitive One Step Polymer ISH Detection Kit will result in an intense stain at the specific site of the hybridized fluorescein-labeled probe in positive test tissue and positive controls. If staining is absent from any positive control slides, or present in any negative control slides, the test should be considered invalid. If deviation from the expected results occurs, please consult the troubleshooting guide of detection systems for assistance.

Limitations of the Procedure

Correct treatment of tissues prior to and during fixation, embedding, and sectioning is important for obtaining optimal results. Inconsistent results may be due to variations in tissue processing, as well as inherent variations in tissue. The results from *in situ* hybridization must be correlated with other laboratory findings.

Performance Characteristics

BioGenex has conducted studies to evaluate the performance of the probe with BioGenex detection systems and accessories. The probes have been found to be sensitive and show specific binding to the antigen of interest with minimal to no binding to non-specific tissues or cells. BioGenex probes have shown reproducible and consistent results when used within a single run, between runs, between lots and wherever applicable between manual and automated runs. The products have been determined to be stable for the periods specified on the labels either by standard real time or accelerated methods. BioGenex ensures product quality through 100% quality control for all products released and through surveillance programs.

Bibliography

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