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HPV 14 Probe
Cat No# PR251-100E
For Manual Staining

Doc. No 932-PR251-100E Rev. D
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Intended Use
The HPV 14 probe is available for in vitro diagnostic use. The probe is designed for the specific detection of 14 HPV genotypes in formalin-fixed paraffin embedded human tissues and cytopathology specimens by *in situ* hybridization.

Summary and Explanation
BioGenex HPV 14 probe has been developed for Chromogenic *In Situ* Hybridization (CISH) assay and facilitates detection of HPV genomes in a morphological context. BioGenex developed oligo probes for the detection of HPV 14 genotypes namely 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 sequences in FFPE tissues and cytopathology specimens/cervical scrapes. Along with the BioGenex Super sensitive™ ISH Detection systems, the HPV 14 probe offers rapid and sensitive detection of nucleic acids with high specificity while preserving tissue morphology.

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 treated with nucleic acid retrieval solution to expose target DNA or mRNA sequences. A hapten (fluorescein 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. (Downstream 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 brown 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 HPV 14 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^{o} C. Warm to room temperature immediately prior to use (HPV probes may need water baths higher than 37^{o} 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 Slide 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 Nucleic Acid Retrieval solution (NAR) following the instruction product data sheet.

Staining procedure
(a) The BioGenex HPV 14 PROBE is used without further dilution.
(b) The probe solution is brought to room temperature just prior to use.
(c) Formalin-fixed, paraffin-embedded tissue sections need pretreatment with Nucleic Acid Retrieval solution (NAR).
(d) The testing parameters and testing protocols are listed in Table below.
(e) The BioGenex Super Sensitive™ ONE STEP POLYMER ISH Detection System (DF400-50KE) is recommended for the staining.
(f) After staining, the slides are dehydrated in 100% reagent alcohol and cleared in xylene.
(g) Permanent mounting medium is applied to the slides.
(h) The negative control probe is run in parallel with the HPV 14 PROBE.

Recommended protocol and parameters for HPV 14 probe

Table 1: Protocol for Manual Staining Procedure -FFPE Cervical Cancer Tissues/Cervical Scrapes/Cell Lines

S.l No	Reagent	Incubation time & temperature
1	Pretreatment-Nucleic Acid Retrieval (NAR)	2 mins @ 85 ^{o}C & 20 mins 102 ^{o}C
2	Probe	10 mins @ 90˚C & 1hr @ 37˚C 1hr
3	Wash Solution A	5 minutes a@ 45^{o}C
4	Wash Solution B	5 minutes @ 55^{o}C
5	Anti mouse HRP	30 mins @ RT

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* Baking step is not required for Cervical Scrapes, cultured cells or cytopathology specimens.
* Cervical Scrapes and Cell Lines requires only 10 minutes of Nucleic Acid Retrieval treatment
* DAB incubation time is 5-10 mins for Cervical Scrapes and Cell Lines

TESTING PARAMETERS
Dispensing pattern : 1/3 (XT014-SL & XT014-CL)
Probe Dispensing volume: 25 µl

Quality Control
The recommended positive control tissue for this probe is cervical cancertissues. 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.

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