48810 Kato Road, Suite 100E & 200E, Fremont, CA 94538,
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In Situ sequencing using NanoVIP ®

INTENDED USE

The *in situ* sequencing technique generates spatially resolved gene expression data for panels of genes at subcellular resolution. The technique has been developed in the lab of Mats Nilsson who has pioneered the field of generating *in situ* gene expression and mutation profiles (Ke R *et al*; 2013 Nature Methods). *In situ* sequencing enables localization and quantification of more than 100 transcripts simultaneously with subcellular resolution in a single tissue section per experiment.

I. PRINCIPLE

The principle of this method involves tissue fixing (to preserve the cell morphology) and subsequent treatment with proteolytic digestion (to provide access for the PCR reagents to the target DNA). The target sequences are amplified by those reagents and then detected by standard immunocytochemical/Immunofluorescence protocols. Formalin fixed paraffin embedded (FFPE) Tissue/cytological specimen on standard microscopy slides are pre-treated to generate cDNA *in situ*. A set of genes are targeted with a custom designed padlock probe library. Probes that have specifically interacted with the targeted transcripts are amplified by Rolling Circle Amplification (RCA) reaction, and the specific amplification generates signals with high signal to noise ratio. The signals are detected by fluorescently labelled oligonucleotide libraries. *In Situ* sequencing can be used to detect up to a few hundred genes per sample. *In Situ* sequencing combines the sensitivity of PCR or RT-PCR amplification along with the ability to perform morphological analysis on the same sample, and thus it is an attractive tool in diagnostic applications. One of the most prominent applications is the detection of infectious disease agents including HIV-1, HBV, HPV, HHV-6, CMV, and EBV.

II. REAGENTS AND MATERIALS

Instrument: The BioGenex **NanoVIP®** is our All-in-One small-footprint systems that are economical, affordable, and ideal for performing *In Situ* Sequencing assays.

Key ingredients: DNA polymerase, Reverse Transcriptase, Fluorescent Labeled dNTPs, Padlock Probes.

Other reagents and ancillaries are listed in BioGenex Product Line:

	Items	Cat: Number
1	XDeWax™	HX015-XAK
2	EZ-AR™ Solution	HX032-04X
3	Peroxide Block	HX026-YADE
4	Power Block	HX083-YADE
5	PEPSIN	HX632-06X
6	eFISH Reagent A	HX972-08X
7	eFISH wash buffer 1	HX604-20X
8	TBS	HK098-YAK
9	PBS	HK091-YAKE
10	1xSSC buffer	HK583-5K
11	Rinse Buffer	HK091
12	Barrier slides	XT128, XT108
13	Coverslips	XT128, XT122

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III. HANDLING, STORAGE AND SHELF LIFE

Precautions: Specimens and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Avoid microbial contamination of reagents to minimize non-specific staining. Wear suitable Personal Protective Equipment. Never pipette reagents by mouth. Avoid contact of reagents and specimens with skin and mucous membranes. If reagents or specimens come into contact with sensitive area, wash with copious amounts of water. Some reagents in this kit contain sodium azide at concentrations of less than 0.1%. Sodium azide is not classified as a hazardous chemical at these concentrations, but proper handling protocols should be observed. For more information on product hazards, precautions and waste disposal, Material Safety Data Sheets are available upon request. Dispose of unused reagents according to Local, State and Federal Regulations. The user is urged to consult the MSDS for this product for further information on product hazards, precautions, and waste disposal. Consult Federal, State, or local regulations for disposal of any potential toxic components.

Storage Conditions: The reagents are to be stored at -20° to 8°C accordingly to storage instruction on individual label.

Expiration: See product labels for expiration dates. Do not use after expiration date stamped on the vial. The performance of the reagents in this kit is backed by the BioGenex Total Quality Assurance policy (see BioGenex Automated Systems Catalog for details).

IV. STEPS INVOLVE IN *IN SITU* SEQUENCING DETECTION SYSTEM

- **Baking of FFPE Tissue Section / Cytological specimen**
- **Dewaxing of FFPE Tissue Section / Cytological specimen**
- **Nucleic Acid Retrival**
- **Protease treatment**
- **Denaturation & Padlock Primer hybridization**
- **Sequencing using fluorescent labeled dNTPs**
- **Signal record using fluorescent detector**

V. STAINING PROCEDURE

Step/Reagent	Reagent	Incubation Time (min)*	Temperature (⁰C)	No. of Washes/ Rinses*	No. of Cycles*
Baking	--	20	70	--	--
Dewaxing	EZ-DeWax™	4	RT	3	3
	DI Water	30 sec	RT	2	1
	Alcohol	2	RT	2	1
	Heat Slide	30 sce	45	--	--
Pre-Treatment	EZ-AR2™ Solution	25min	95-100	1	--
	eFISH Wash Buffer 1	1 sec	RT	2	1
	DI water	3min	RT	2	1
Pepsin Digestion	eFISH Liquid Pepsin	10-20	37	1	1
	eFISH Wash Buffer 1	30sec	RT	--	--
	DI Water	30 sec	RT	2	1
	Alcohol	2	RT	2	1
DNase treatment (RNAse free)	DNase treatment	45 min	37	1	1

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Primer addition & sequencing	MasterMix (Padlock Primes, Fluorescent dNTPs, Reverse Transcriptase)	1 min 1 min	94 65	-- --	-- --
Signal Record	Signal Record	--	--	--	--
wash	DI Water	10 sec	RT	2	1
Repeat the steps Primer addition, sequencing & Signal Record					

LIMITATIONS

It is recommended that the reagents not be substituted across kit lot numbers. Interpretation of the staining result is solely the responsibility of the user. Experimental results should be confirmed by a medically established diagnostic product or procedure. Evaluation must be performed by a qualified pathologist. Improper tissue handling and processing prior to immunostaining can lead to inconsistent results. Variations in embedding and fixation or the nature of the tissue may lead to variations in results. Endogenous peroxidase activity or pseudo peroxidase activity in erythrocytes and tissue biotin may result in non-specific staining based on the detection system employed. Improper counterstaining and mounting may compromise the interpretation of results. Normal/non-immunesera from the same animal source as secondary antisera used in blocking steps may cause false negative or-positive results due to natural or auto-antibodies.

REFERENCES

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