Seraseq™

HIV-1 Drug Resistance and Tropism Reference Material Kit v2 (MF1-20)

Package Insert

PLEASE NOTE:
THESE REAGENTS MUST NOT BE SUBSTITUTED FOR THE MANDATORY POSITIVE AND NEGATIVE CONTROL REAGENTS PROVIDED WITH MANUFACTURED TEST KITS.

NAME AND INTENDED USE

The Seraseq™ HIV-1 Drug Resistance and Tropism Reference Material Kit v2 (MF1-20) is intended for use with HIV-1 Next Generation Sequencing (NGS) assays that identify drug resistance mutations or assess the gene sequence for the GP120 variant loops to determine tropism. The Seraseq HIV-1 Drug Resistance and Tropism Reference Material uses the AccuPlex™ recombinant viral technology and evaluates detection of 49 mutations across gag, protease (PRO), reverse transcriptase (RT), and integrase (INT) regions (Table 1). The reference material also contains envelope sequences indicative of either CCR5 or CXCR4 co-receptor usage and can be used to monitor tropism determination.

The Seraseq HIV-1 Drug Resistance and Tropism Reference Material Kit v2 (MF1-20) is intended for use by diagnostics manufacturers, researchers, and clinical laboratories to develop, evaluate, monitor or troubleshoot NGS HIV-1 drug resistance assays. *For Research Use Only. Not for use in diagnostic procedures.*

SUMMARY

Assay developers and researchers rely on positive disease state materials that can effectively challenge all elements of an assay from sample preparation through amplification and detection. Valuable reference materials will mimic natural samples and go through extraction, reverse transcription, sequencing and data analysis. Seraseq HIV-1 Drug Resistance and Tropism Reference Material Kit v2 (MF1-20) consist of enveloped viral particles in a commutable plasma-based matrix and can be used as a process reference material.

Use of reference materials as part of a well-designed quality control program can provide added confidence in the reliability of results obtained for unknown specimens. The use of independent controls and reference materials may provide valuable information concerning laboratory proficiency and kit lot variation that may affect assay Sensitivity[1].

PRINCIPLES OF THE PROCEDURE

Seraseq HIV-1 Drug Resistance and Tropism Reference Material Kit v2 (MF1-20) is intended for use with NGS assays that identify minority drug resistance mutations. The reference material is a mixture of recombinant viruses and contains variants in the Gag/PRO/ RT/ INT and Envelope regions at 3 different mutation frequencies (target of 1%, 5%, and 20%). The variants in the reference material were selected based on their key role in antiretroviral drug resistance[2]. The reference material contains Gag/PRO/RT/INT regions which correspond to nucleotides 1400-5400 in HXB2 reference strain[3] (Genbank K03455) and envelope regions which correspond to 6300-7825.

The reference materials should follow the same workflow as test samples and require viral nucleic acid extraction prior to use in NGS assays. Seraseq HIV-1 Drug Resistance and Tropism Reference Material Kit v2 (MF1-20) utilizes AccuPlex technology in which an RNA containing enveloped Alpha virus is engineered to contain the desired HIV-1 sequences. The resulting recombinant viral particles are efficiently packaged in host cells, but lack the genes required to produce new viral particles, and are therefore replication defective[4].

REAGENTS

Item No. 0740-0026. 3 vials, 1 mL per vial, 5.0E+04 viral copies/mL concentration.

Name	Mutant Frequency Target
MF-20	20%
MF-5	5%
MF-1	1%

WARNINGS AND PRECAUTIONS

For Research Use Only. Not for use in diagnostic procedures.
CAUTION: Handle Seraseq HIV-1 Drug Resistance and Tropism Reference Material Kit v2 (MF1-20) and all materials derived from human blood products as though they are capable of transmitting infectious agents. Seraseq HIV-1 Drug Resistance and Tropism Reference Materials are formulated in a plasma-based matrix, tested and found negative for common bloodborne pathogens (negative for the presence of antibody to HIV1/2, antibody to HCV, antibody to HBc, antibody to HTLV I/II, HIV RNA, HCV RNA, HBV DNA and HBsAg).

Safety Precautions

Use Centers for Disease Control and Prevention (CDC) recommended universal precautions for handling reference materials and human specimens[5]. Do not pipette by mouth; do not smoke, eat, or drink in areas where specimens are being handled. Clean any spillage by immediately wiping up with 0.5% sodium hypochlorite solution. Dispose of all specimens and materials used in testing as though they contain infectious agents.

Handling Precautions

Do not use Seraseq HIV-1 Drug Resistance and Tropism Reference Material Kit v2 (MF1-20) beyond the expiration date. Avoid contamination of the product when opening and closing the vials.

STORAGE INSTRUCTIONS

Store Seraseq HIV-1 Drug Resistance and Tropism Reference Material Kit v2 (MF1-20) frozen at -20°C or colder. Once thawed and opened, an individual vial of Seraseq HIV-1 Drug Resistance and Tropism Reference Material Kit v2 (MF1-20) should not be reused.

INDICATIONS OF REAGENT INSTABILITY OR DETERIORATION

Seraseq HIV-1 Drug Resistance and Tropism Reference Material Kit v2 (MF1-20) is a mixture of recombinant Alpha viruses that bear HIV-1 sequences suspended in a plasma-based matrix. It will appear as a yellow to amber liquid. Alterations in this appearance or visible microbial growth may indicate instability or deterioration of the product and vials should be discarded.

PROCEDURE

Materials Provided

Seraseq HIV-1 Drug Resistance and Tropism Reference Material Kit v2 (MF1-20) is a mixture of recombinant viruses that bear HIV-1 Gag/Protease/ Integrase/ RT/ Envelope sequences suspended in a plasma-based matrix. One (1) mL is provided per tube and the concentration is approximately 5.0E+04 viral copies/mL as determined by a digital PCR assay.

Materials Required but not Provided

Refer to instructions supplied by manufacturers of the test kits to be used.

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Instructions for Use

Allow the vial to come to room temperature before use. Mix by vortexing to ensure a homogeneous suspension. Seraseq HIV-1 Drug Resistance and Tropism Reference Material Kit v2 (MF1-20) should be added to a test run using the same procedure provided by the manufacturer for unknown specimens. Seraseq HIV-1 Drug Resistance and Tropism Reference Material Kit v2 (MF1-20) must go through an extraction process prior to library preparation and NGS sequencing. Refer to your usual assay procedures in order to determine the amount of material to use.

Quality Control

Although Seraseq HIV-1 Drug Resistance and Tropism Reference Material Kit v2 (MF1-20) is offered in a target mutation frequency of 20%, 5% and 1%, the product does not have assigned values for the drug resistant mutations or mutation frequencies. There are many reasons why assays may observe deviation from the representative data which may or may not be of significance. It is therefore recommended that each laboratory qualify the use of Seraseq HIV-1 Drug Resistance and Tropism Reference Material Kit v2 (MF1-20) with each assay system prior to its routine use.

INTERPRETATION OF RESULTS

Detection of the variants within Seraseq HIV-1 Drug Resistance and Tropism Reference Material Kit v2 (MF1-20) may vary with different types of tests and different test kit lots. Since the reference material does not have an assigned value, the laboratory must establish a range for each lot of Seraseq HIV-1 Drug Resistance and Tropism Reference Material Kit v2 (MF1-20). When results for the product are outside of the established acceptance range, it may indicate unsatisfactory test performance. Possible sources of error include: deterioration of test kit reagents, operator error, faulty performance of equipment, contamination of reagents or change in bioinformatics pipeline parameters.

LIMITATIONS OF THE PROCEDURE

Seraseq HIV-1 Drug Resistance and Tropism Reference Materials Kit v2 (MF1-20) MUST NOT BE SUBSTITUTED FOR THE CONTROL REAGENTS PROVIDED WITH MANUFACTURED TEST KITS. *TEST PROCEDURES* and *INTERPRETATION OF RESULTS* provided by manufacturers of test kits must be followed closely. Deviations from procedures recommended by test kit manufacturers may produce unreliable results. Seraseq HIV-1 Drug Resistance and Tropism Reference Materials Kit v2 (MF1-20) are not calibrators and should not be used for assay calibration. Adverse shipping and storage conditions or use of outdated product may produce erroneous results.

EXPECTED RESULTS

Specific detection of variants and variant allele frequencies will vary among different assays, different procedures, different lot numbers, and different laboratories. Each laboratory should establish its own range of acceptable values. For example, the acceptable range might include all values within 2 standard deviations of the mean of 20 data points obtained in 20 runs over a period of 20 days[6]. Table 1 lists key mutations that are present in the product.

SPECIFIC PERFORMANCE CHARACTERISTICS

Seraseq HIV-1 Drug Resistance and Tropism Reference Materials Kit v2 (MF1-20) have been designed for use with NGS sequencing procedures for the purposes of assessing assay performance. Seraseq HIV-1 Drug Resistance and Tropism Reference Materials do not have assigned values. Procedures for implementing a quality assurance program and monitoring test performance on a routine basis must be established by each individual laboratory.

REFERENCES

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2. Johnson VA, Calvez V, Günthard HF, Paredes R, Pillay D, Shafer RW, Wensing AN, and Richman DD. 2013. Update of the Drug Resistance Mutations in HIV-1: March 2013 Topics in Antiviral Medicine. Volume 21 Issue 1 pp 6-14.
3. http://www.hiv.lanl.gov/content/sequence/HIV/MAP/annotation.html
4. Schlesinger S. 2000. *Alphavirus expression vectors.* Adv Virus Res. 55:565-77.
5. Siegel JD, Rhinehart E, Jackson M, Chiarello L, and the Healthcare Infection Control Practices Advisory Committee, 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings.
6. Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline-Second Edition. NCCLS document C24-A2, 1999.

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Table 1: List of Mutations Incorporated

Gag	Protease Gene	RT Gene (NRTI)	RT Gene (NNRTI)	Integrase Gene
H219Q (CAT to CAA)	L24I (TTA to ATA)	M41L (ATG to TTG)	L100I (TTA to ATA)	T66I (ACA to ATA)
G381S (GGC to AGC)	D30N (GAT to AAT)	K65R (AAA to AGA)	K101E (AAA to GAA)	L74M (CTG to ATG)
	V32I (GTA to ATA)	D67N (GAC to AAC)	K103N (AAA to AAC)	E92Q (GAA to CAA)
	M46I (ATG to ATA)	T69S (ACT to TCT) with the insertion of two additional Seriens (TCCTCC)	V108I (GTA to ATA)	T97A (ACA to GCA)
	I47L (ATA to CTA)	K70R (AAA to AGA)	V106A (GTA to GCA)	E138A (GAA to GCA)
	G48V (GGG to GTG)	L74V (TTA to GTA)	Y181C (TAT to TGT)	G140S (GGA to TCA)
	I50V (ATT to GTT)	F77L (TTC to CTC)	Y188L (TAT to TTA)	Y143R (TAC to CGC)
	I54M (ATC to ATG)	Y115F (TAT to TTT)	G190A (GGA to GCA)	Q148H (CAA to CAC)
	G73S (GGT to TCT)	F116Y (TTT to TAT)	P225H (CCT to CAT)	N155H (AAT to CAT)
	L76V (TTA to GTA)	Q151M (CAG to ATG)	M230L (ATG to CTG)	
	V82A (GTC to GCC)	M184V (ATG to GTG)		
	I84V (ATA to GTA)	L210W (TTG to TGG)		
	N88D (AAT to GAT)	T215Y (ACC to TAC)		
	L90M (TTG to ATG)	K219Q (AAA to CAA)		