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GenomeLab™ GeXP Human Reference*Plex* Kit

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

The GenomeLab GeXP Human Reference*Plex* Kit (PN A54657) contains reagents used to monitor the expression of the following genes of interest represented in human RNA samples.

Note: *For RNA isolation, see the Agencourt RNA preparation protocols.*

Number	Target Gene	ID	Size*
1	Ezrin	X51521	150
2	QRSHs glutaminyl-tRNA synthetase	X76013	160
3	Histone deacetylase HD1	U50079	165
4	Transferrin Receptor	BC001188	172
5	Nuclear factor NF45	U10323	186
6	MLN51	X80199	197
7	glycerol kinase	NM_203391	201
8	Proteasome subunit Y	D29012	211
9	Ribosomal protein L37a (RPL37A)	L06499	214
10	18s-rRNA	M10098	220
11	E2 Ubiquitin conjugating enzyme UbcH5B	U39317	225
12	Elongation factor EF-1-alpha	NM_001402	233
13	cyclophilin A	BC000689	237
14	Lysosomal hyaluronidase	AJ000099	253
15	Transcription Factor IID	X97999	258
16	beta-actin	NM_001101	267
17	GAPDH	NM_002046	277
18	ATP synthase	X83218	281
19	18kDa Alu RNA binding protein (SRP14)	NM_003134	291
20	Hypoxanthine ribosyl transferase	M31642.1	305
21	Beta 2 microglobulin	NM_004048	314
22	Ca2-activated neutral protease large subunit	M23254	317
23	Kanamycin resistance**	n/a	325
24	Acidic Ribosomal Protein (RPLP0)	NM_001002	330
25	beta-glucuronidase	NM_000181	338

**The size listed is the Expected CE Size which is instrument dependent. Perform several control runs on each GenomeLab GeXP System to identify the Expected CE Size for each gene on each instrument, and follow instructions in the GenomeLab User's Guide (A29142) for setting up Locus Tag and Allele IDs using the Fragment Analysis Software module.*

***RNA from an external source (see KAN^r RNA with RI Reagent in the GenomeLab GeXP Start Kit insert (A85019) for more information) used as an internal control.*

Kit Contents

- RT Rev Primer Plex Human Reference*Plex*, 240 µL (orange)
- PCR Fwd Primer Plex Human Reference*Plex*, 240 µL (blue)
- Control RNA Templates Human Reference*Plex*, 30 µL at 100 ng/µL (from human source) (gray)

Note: *To order reagents, use Kit Reorder No. A54657.*

Materials Required but not Supplied with this Kit

Reagents

- GenomeLab GeXP Start Kit (A85017)
- Thermo-Start® DNA Polymerase* with separate 25 mM $MgCl_2$ (A85022)
- Nuclease-Free H_2O, non-DEPC Treated (Affymetrix 71786 or Thermo Fisher Scientific, Inc. 10977-015)
- 1M Tris-HCl pH 8.0 (Affymetrix 22638)
- The RNA Storage Solution (Thermo Fisher Scientific, Inc. AM7000)
- GenomeLab Separation Buffer (608012)
- GenomeLab Separation Capillary Array (608087)
- GenomeLab Separation Gel, 20 mL (391438) for GeXP dual-rail systems, or 10 mL (608010) for GeXP single-rail systems

**Manufactured by Thermo Fisher Scientific; capable of 100 reactions.*

Equipment and Supplies

- GenomeLab GeXP Genetic Analysis System (A26572 or A62684)
- Sample Microplates, 96-Well (609801)
- 8-Well Cap Stops (BioRad TCS-0803)
- Buffer Microplates, 96-Well (609844)
- 1.5 mL and 0.65 mL Microtubes
- Pipettors, P10, P20, P100, P200, and P1000
- Aerosol Resistant Tips for P10, P20, P100, P200 and P1000
- Microtube Centrifuge
- Microplate Centrifuge
- Thermocycler with Heated Lid for 96-Well Plates
- Vortex Mixer
- Non-Frost-Free Freezers (-80°C and -20°C)

Preparation and Storage

Control RNA Templates

Store at -80°C to -65°C in non-frost-free freezer.

All Other Reagents

Store at -35°C to -15°C in non-frost-free freezer.

Aliquoting

Control RNA Templates

When using the Control RNA Templates for the first time, thaw the RNA Templates, (see "Thawing Control RNA Templates" below) then aliquot in 2.5 µL aliquots. Store aliquots at -80°C to -65°C in a non-frost-free freezer.

Note: *Before using the 2.5 µL Control RNA Templates aliquot, thaw, then dilute with 47.5 µL DNase/RNase Free H_2O to 5 ng/µL. Use each aliquot only once. Do not re-freeze/thaw the Control RNA Templates aliquots.*

Thawing Primer Plexes

Before use, thaw the reagent at room temperature (+20°C to +25°C) for 30 minutes without opening the tube. After thawing is complete, mix the reagent by gently inverting 10 to 15 times. Do a quick centrifuge spin to consolidate the solutions to the bottom of the tube.

Thawing Control RNA Templates

Thaw the RNA Templates at room temperature for 5 minutes, without opening the tube. After thawing is complete, do a quick centrifuge spin to consolidate the solution to the bottom of the tube.

Note: *Do not thaw reagents at temperatures above 25°C.*

Handling Precautions

Please be aware of the following handling precautions. For detailed information, see 67-548-EEC (Directive on Dangerous Substances), 88-379-EEC (Dangerous Preparations Directive) and 21 CFR 1910.1200 (USA OSHA Hazard Communications).

Protocol

Refer to the GenomeLab GeXP Chemistry Protocol (A29143) for detailed instructions on using this kit. This protocol is included with the system or available from www.beckmancoulter.com.

RT Reaction

1. Assemble the RT Reaction mixture according to the table below and add the reaction mixture to the appropriate wells of a new 96-Well Sample Microplate (609801) - the RT Plate:

RT Reaction Mix	Volume Per Well
DNase/RNase Free H_2O	3 µL
RT Buffer 5X	4 µL
RT Rev Primer Plex	2 µL
Reverse Transcriptase*	1 µL
Pre-diluted KAN^r RNA with RI#	5 µL
Sample RNA (5-20 ng/µL) or diluted Control RNA Templates (5ng/µL)**	5 µL (25-100 ng total)
Total	**20 µL**

**For RT-Minus control, substitute DNase/RNase Free H_2O for Reverse Transcriptase.*

 # Dilute KAN^r RNA in 10 mM Tris-HCL, pH 8 or RNA Storage Solution, aliquot and store at -80°C. A 1:50 pre-dilution is recommended for this multiplex.

***For no-template control, substitute DNase/RNase Free H_2O for Sample RNA or Control RNA Templates.*

2. Run the following incubation program:

Step	Temp	Time
1	48°C	1 minute
2	42°C	60 minutes
3	95°C	5 minutes
4	4°C	Hold

PCR Reaction

1. Assemble the PCR Reaction mixture according to the table below and add the reaction mixture to the appropriate wells of a new 96-Well Sample Microplate - the PCR Plate:

PCR Reaction Mix	Volume per Well
PCR Buffer 5X	4.0 µL
25 mM $MgCl_2$ (Thermo-Start)	4.0 µL
PCR Fwd Primer Plex	2.0 µL
Thermo-Start DNA Polymerase (A85022)	0.7 µL
cDNA Samples (RT reactions from the RT Plate)	9.3 µL
Total	**20.0 µL**

2. Run the following thermal cycler program:

Step	Temp	Time
1	95°C	10 minutes
2	94°C	30 seconds
3	55°C	30 seconds
4	70°C	1 minute
5	N/A	Repeat steps 2-4 for an additional 34 cycles (total of 35 cycles)
6	4°C	Hold

Pre-Dilution

1. Prepare 10 mM Tris-HCl pH 8.0 from 1M Tris-HCl pH 8.0 (Affymetrix 22638) and Nuclease-Free H_2O (Affymetrix 71786) at a ratio of 1:99 (v/v).
2. Assemble the following mix and add it to the appropriate wells of a new 96-Well Sample Microplate - the Pre-dilution Plate:

Pre-Dilution Mix	Volume per Well
PCR Reaction Samples from the PCR Plate	2 µL
10 mM Tris-HCl pH 8.0	8 µL*
Total	**10 µL**

**Additional 10 mM Tris-HCl pH 8.0 can be added to optimize the sample Pre-dilution concentration.*

GenomeLab Sample Plate Setup

Assemble the following mix and add it to the appropriate wells of a new 96-Well Sample Microplate - the Sample Plate:

GenomeLab Sample Mix	Volume per Well
Diluted PCR Reaction Samples from the Pre-dilution Plate	1.0 µL
DNA Size Standard-400	0.5 µL
Sample Loading Solution	38.5 µL
Total	**40.0 µL**
Mineral Oil	1 drop

GenomeLab Buffer Plate Setup

Fill the appropriate number of columns of a new 96-Well Buffer Microplate (609844) with approximately 250 µL of GenomeLab Separation Buffer (608012) - the Buffer Plate.

GenomeLab GeXP Genetic Analysis System

Refer to the GenomeLab User's Guide (A29142) for detailed instructions on running samples with the GenomeLab GeXP System, using the Fragment Analysis software module, the GeXP Data Tool, the GeXP Quant Tool and for performing GeXP gene expression profiling analysis.

Running Samples on the GenomeLab GeXP System

1. Launch the Plate Setup module on the GenomeLab GeXP System controller and set up a plate to run the samples using the default **Frag-3** protocol.
2. Load the Sample Plate(s) and the Buffer Plate(s) and start the GenomeLab GeXP System.

Fragment Analysis

1. Select **DefaultGeXPAnalysisParameters** when analyzing the data obtained from the GenomeLab GeXP System.

***Note:** Edit the DefaultGeXPAnalysisParameters when using the Standard Curve GeXP Quantitative Analysis Method. Change the **Slope Threshold** to one (1) and **Relative Peak Height Threshold** to zero (0) %. Save as "**GeXP Sensitive**" analysis parameters. Use this set of analysis parameters for fragment analysis of standard curve and experimental samples.*

2. Set up and apply Locus Tag and Allele IDs through binning following instructions in the GenomeLab User's Guide (A29142).
3. Use the following Exclusion Filter in the **Study-Data-Fragments List** view to exclude untagged fragments from the sample result.

ID	Name	Operator	Value
1	allele ID	=	

4. From the **File** menu, select **"Transfer Fragments for GeXP**..." and export the GeXP fragment data as a .csv file for further analysis in the GeXP Data Tool and the GeXP Quant Tool.

GeXP Data Tool

1. Download the GeXP Data Tool from the Beckman Coulter website (www. beckmancoulter.com/genomelab).
2. Select "Open" and Choose a GeXP data file (in .csv format) to import to GeXP Data Tool.
3. Checkmark the box next to "Normalization". KAN^r is selected as the default normalization gene for the GeXP Quantitative Analysis Method (Standard Curve Method).
4. Export the normalized data set as a text (.txt) file.

GeXP Quant Tool

Perform sample analysis against a standard curve using the text file(s) with the GeXP Quant Tool software. Download the GenomeLab GeXP Quant Tool from the Beckman Coulter website (www.beckmancoulter.com/genomelab).

250 S. Kraemer Blvd., Brea, CA 92821 U.S.A.
Visit us at www.beckmancoulter.com