A85019AA
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GenomeLab™ GeXP Start Kit

FOR LABORATORY USE ONLY

The GenomeLab GeXP Start Kit (PN A85017) contains reagents needed to perform multiplex assays for monitoring the expression of genes of interest present in RNA samples. A custom multiplex can be designed for the detection of up to 30 genes.

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

Kit Contents

- RT Buffer 5X, 480 µL (green)
- Reverse Transcriptase, 120 µL at 20 units/µL (red)
- PCR Buffer 5X, 480 µL (amber)
- KAN^r RNA with RI, 600 µL (yellow)
- DNase/RNase Free H_2O, 1200 µL (white)
- DNA Size Standard-400, 55 µL
- Mineral Oil, 5 mL
- GenomeLab Sample Loading Solution, 6 mL

Note:*To order reagents, use Kit Reorder No. A85017.*

Materials Required but not Supplied with this Kit

Reagents

- Thermo-Start® DNA Polymerase with separate 25 mM $MgCl_2$ (PN A85022)
- Nuclease-Free H_2O, non-DEPC Treated (USB 71786 or Invitrogen 10977-015)
- 1 M Tris-HCl pH 8.0 (USB 22638)
- The RNA Storage Solution (Applied Biosystems AM7000)
- GenomeLab Separation Buffer (PN 608012)
- GenomeLab Separation Capillary Array (PN 608087)
- GenomeLab Separation Gel, 20 mL (PN 391438), or 10 mL (PN 608010) for single-plate systems such as CEQ 8000.

Equipment and Supplies

- GenomeLab GeXP Genetic Analysis System (PN A26572 or PN A28130)
- Sample Microplates, 96-Well (PN 609801)
- 8-Well Cap Stops (BioRad TCS-0803 or Corning/Costar 0556)
- Buffer Microplates, 96-Well (PN 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 (PN 365603)
- Microplate Centrifuge
- Thermocycler with Heated Lid for 96-Well Plates
- Vortex Mixer
- Non-Frost-Free Freezers (-80°C and -20°C)

Preparation and Storage

KAN^r RNA with RI
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.

Note: *Size Standard-400 and PCR Buffer 5X are photosensitive. Avoid exposure to light.*

Thawing

PCR Buffer 5X
Frozen PCR Buffer 5X may contain harmless precipitation. Thawing and thorough mixing are required to redissolve any precipitation back into solution. Before use, thaw the reagent at room temperature (+20°C to +25°C) for 30 minutes without opening the tube. After complete thawing, do a quick centrifuge spin then mix the reagent by pipetting deeply into the inner tube bottom 5 to 10 times.

Reverse Transcriptase
Reverse Transcriptase is in a glycerol solution. Thawing is not required. Before use, do a quick centrifuge spin to release any solution from the cap and tube wall. Pipet this solution slowly and carefully. The viscosity of the glycerol in the enzyme solution can lead to pipetting errors.

All Other Reagents
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 release any solution from the cap and tube wall.

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

Handling Precautions

Please be aware of the handling precautions listed below. 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).
Toxic. Contains Formamide. R61 May cause harm to unborn child. R36/37 Irritating to eyes and respiratory system. S24/25 Avoid contact with skin and eyes. S37 Wear suitable gloves. S45 In case of accident, or if you feel unwell, seek medical advice immediately. S53 Avoid exposure-obtain special instructions before use.

Primer Design

Use the eXpress Designer module of the eXpress Profiler software to design multiplex primer sets. Refer to the GenomeLab User's Guide (PN A29142) and the GenomeLab GeXP Chemistry Protocol (PN A29143) for detailed instructions on multiplex primer design.

Protocol

Refer to the GenomeLab GeXP Chemistry Protocol (PN A29143) for detailed instructions for using this kit. This manual 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 - the RT Plate:

RT Reaction Mix	Volume Per Well
DNase/RNase Free H_2O	3 µL
RT Buffer 5X	4 µL
Custom 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)***	5 µL (25-100 ng total)
Total	**20 µL**

** Customers need to design and prepare this primer plex.*

***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. Optimization of the KAN^r RNA concentration is necessary for a custom multiplex. An initial dilution of 1:50 is recommended.

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

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 (PN 609801) – the PCR Plate:

PCR Reaction Mix	Volume per Well
PCR Buffer 5X	4.0 µL
25 mM $MgCl_2$ (Thermo-Start)	4.0 µL
Custom PCR Fwd Primer Plex*	2.0 µL
Thermo-Start DNA Polymerase (A25395)	0.7 µL
cDNA Samples (RT reactions from the RT Plate)	9.3 µL
Total	**20.0 µL**

** Customers need to design and prepare this primer plex.*

2. Run the following thermal cycling 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

Depending on the sample concentration, the following can be made for pre-dilution.

1. Prepare 10 mM Tris-HCl pH 8.0 from 1 M Tris-HCl pH 8.0 (USB 22638): Nuclease-Free H_20 (USB 71786) = 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 Setup

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

GenomeLab Sample Mix	Volume per Well
PCR Reaction Samples (undiluted from the PCR Plate or diluted 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 (PN 609844) with approximately 250 µL of GenomeLab Separation Buffer (PN 608012) – the Buffer Plate.

GenomeLab GeXP Genetic Analysis System

Refer to the GenomeLab User's guide (PN A29142) for detailed instructions on running samples with the GenomeLab GeXP System, using the Fragment Analysis software module, the eXpress Profiler software, the eXpress Analysis software module and for performing GeXP gene expression profiilng analysis.

Running Samples on the GenomeLab GeXP Genetic Analysis 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 run.

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. Use the following Exclusion Filters in the **Study-Data-Fragments List** view to exclude unwanted fragments from the sample result.

ID	Name	Operator	Value
1	dye	Not Equal	D4
2	est frg size (nt)	<	130
3	est frg size (nt)	>	400

3. From the **File** menu, select **Transfer Fragments for GeXP** and export the plate fragments .csv file for eXpress Profiler analysis.

eXpress Analysis

1. Select the customer designed multiplex of interest in GeXP Analysis Setup.
2. Import the plate fragments .csv file in GeXP Import.
3. Associate plate samples with the customer designed multiplex of interest for the Analysis in the Sample Layout tab.
4. Perform peak binning to align the designed gene peak sizes with actual data peaks.
5. Choose KAN^r as the normalization gene for the GeXP Quantitative Analysis Method (Standard Curve method).
6. Export the normalized data set as a **Profile (by Gene)** report in text (.txt) format.
7. 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).

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