Certificate of Analysis & Product Manual

qPCR, Gene Expression, SNP Analysis, Mutation Detection, Human Identification,
Forensic Casework, Sample Collection
Trace DNA Extraction, STR Analysis
DNA Biometrics, Q-PCR

GeneAssays™ Genotyping PCR 2X Master Mix

Catalog No.: 41-1031-XX & 41-1036-XX

Storage Condition: Store at -20°C to -10°C

For Research Use Only. Not for use in diagnostic procedures for clinical purposes

2X Master Mix with and without Rox reference dye
Convenient Ready to Use 2X Concentration
Hot Start Taq Polymerase

Applications
Genotyping Assays
Real Time Quantitative PCR Analysis (qPCR)
SNP Allelic Assay
Fluorescent Genotyping Assay
siRNA Gene Knockout Validation
Allelic Discrimination
Antisense Targeting
Aptamer Detection Probes

Material Supplied

Storage Condition: Store at -20°C to -10°C. For regular daily use and to avoid multiple freeze thaws the master mix can be stored at 4°C for ~30 days after first use.

GeneAssays™ Genotyping PCR 2X Master Mix			
Content	Catalog No.	Product	Size
GeneAssays™ Genotyping PCR 2X Master Mix Without Reference Dye			
□	41-1031-01	GeneAssays™ Genotyping PCR 2X Master Mix without ROX; 1 mL, 100 rxns.	1 mL
□	41-1031-10	GeneAssays™ Genotyping PCR 2X Master Mix without ROX; 10X 1 mL, 1000 rxns.	10 mL
GeneAssays™ Genotyping PCR 2X Master Mix With ROX Reference Dye			
□	41-1036-01	GeneAssays™ Genotyping PCR 2X Master Mix with ROX; 1 mL, 100 rxns.	1 mL
□	41-1036-10	GeneAssays™ Genotyping PCR 2X Master Mix with ROX; 10X 1 mL, 1000 rxns.	10 mL

Certificate of Analysis & Product Specifications

All component reagents have been manufactured using molecular biology grade water and certified to be DNase & RNase Free. Taq Polymerase is antibody bound for activation after hot start PCR cycle. The 2X Master Mix components include buffer, dNTP, $MgCl_2$ and other proprietary reaction components that enhance steady consistent amplification from less than 10 pg DNA. Each lot is tested for human male genomic DNA dilution curve qQPCR with RNase P and PGR (progesterone receptor) probes.

Appropriate nuclease free handling, dispensing and storage conditions required.

Manufacturing lot numbers are stated on the label of each product and accompanying packing slip.

Product Label Information

	Catalog Number	Description	Size
REF	41-1031-01	GeneAssays™ Genotyping PCR 2X Master Mix without ROX; 1 mL, 100 rxns.	1 mL
	41-1031-10	GeneAssays™ Genotyping PCR 2X Master Mix without ROX; 10X 1 mL, 1000 rxns.	10 mL
	41-1036-01	GeneAssays™ Genotyping PCR 2X Master Mix with ROX; 1 mL, 100 rxns.	1 mL
	41-1036-10	GeneAssays™ Genotyping PCR 2X Master Mix with ROX; 10X 1 mL, 1000 rxns.	10 mL

RUO Research Use Only	-10°C -20°C	LOT
Research Use Only	**Storage** Store at -20°C to -10°C	**Lot Number** Stated on product tube and packing slip
		
Expiry One year from date of shipment	**Instructions** Consult product manual	**QR Code** Visit Gene Link website for product details

IMPORTANT NOTE

1. GeneAssays™ Genotyping 2X PCR master mix can be used for all fluorescent probe assays including TaqMan®, Molecular Beacon and non-probe based assays.
2. Gene Link also manufactures custom probes and primers. Consult custom probe and primers product specification sheet and material supplied for specifications of product received.
3. This product guide is not specific to any particular fluorescent molecular probe assay mix.
4. This product guide should be used in conjunction with the particular instrument manual and specifications and is not intended to replace those specifications. Consult instrument manufacturer for specific details.

Product Description & Application

GeneAssays™ Genotyping PCR 2X Master Mix Without Reference Dye (Catalog No.: 41-1031-XX)
For use with the following real time thermal cyclers that do not require passive reference dye. The list is not comprehensive; please refer to instrument manufacturer for passive dye requirements and specifications
- Bio-Rad. CFX, iQ™, and Opticon™ Real-Time PCR Detection Systems
- Roche. LightCycler® Real-Time PCR Systems.
- All thermal cyclers for standard hot start PCR.

GeneAssays™ Genotyping PCR 2X Master Mix With ROX Reference Dye (Catalog No.: 41-1036-XX)
For use with the following real time thermal cyclers that requires passive reference dye. The list is not comprehensive; please refer to instrument manufacturer for passive dye requirements and specifications
-Thermo Fisher. 7500, 7500HT, 7300, QuantStudio™, StepOne™ and StepOnePlus™
- Agilent. Mx3005P™ and Mx400P™.

The GeneAssays™ Genotyping PCR 2X Master Mix is a convenient hot start PCR mix optimized for fluorescent probe based qPCR, gene expression, allelic discrimination, single nucleotide polymorphism (SNP) applications and can also be used for standard end point PCR quantitation with Sybr® Green and other dyes.
The GeneAssays™ Genotyping PCR 2X Master Mix is supplied in two different formulations for use without reference dye or with ROX as the passive reference dye.

Gene Link also custom synthesizes molecular probe assay for gene expression, SNP analysis or gene detection of any nucleic acid whose sequence is known can be designed, synthesized and custom formulated as a primer/probe mix by Gene Link. Our online GeneAssays™ design page provides a convenient tool for designing and placing orders. Fluorescent molecular probe assay mix are designed and synthesized by Gene Link with a wide array of fluorophores and quenchers. All TaqMan and Molecular Beacon probes with the primers are supplied in various standard formats or can be custom formulated with differing concentration of primers and probes.

Template DNA Requirements

The GeneAssays™ Genotyping PCR 2X Master Mix has been validated with less than 10 pg of template DNA. It is not recommended to use more than 100 ng of template DNA. The DNA can be added as a dissolved solution or pre-delivered and then dried down sample in the wells or tubes.

Control qPCR Assay Mix

A control qPCR assay mix is valuable when starting out with a new custom probe assay mix and for quantifying template DNA. Gene Link provides validated qPCR assay mix for human RNase P and PGR targets. The assay mix contains primers and probe that are validated.

Protocol

A. Advance Preparation

1. Calculate the number of reactions you plan to perform and add 10% more for the preparation of reaction premixture to account for pipetting volume loss.
2. Determine the final volume of PCR reaction (5, 10 or 20 µL) to perform.
3. Always add positive controls and no template controls (NTC) on each plate.
4. Thaw the GeneAssays™ Genotyping PCR 2X Master Mix if frozen and keep on ice.
5. Have the primer mix and probe assay mix ready. Calculate the amount to be added. The table below suggests to use a 10X Primer/Probe assay mix with a 1X final concentration of 500 nM ((0.5 µM each (0.5 pmol/µL) primers and 250 nM (0.25 µM each (0.25 pmol/µL) of probe for standard qPCR. For SNP analysis with more than one probe the probe concentration for each probe is 250 nM (0.25 µM each (0.25 pmol/µL). Follow your optimized primer/probe concentration or other source as required. See appendix for details.
6. All components should be vortexed after thawing and centrifuged briefly.
7. Program thermal cycler/real time PCR thermal cycler for the file/program to be used.

B. Reaction Premix Preparation Worksheet (prepare 10% more than required)

DO NOT ADD TEMPLATE DNA TO THE PREMIX. The template DNA row is only for calculation of the total volume to be added and thus the volume of nuclease free water to be added to arrive at the final volume of the reaction.

Component	/5 µLrxn	Worksheet	/10 µLrxn	worksheet	/20 µLrxn	worksheet
GeneAssays™ Genotyping PCR 2X Master Mix (with or w/o Rox)	2.5 µL		5 µL		10 µL	
10X Primers & Probe(s) assay mix	0.5 µL		1 µL		2 µL	
*Nuclease Free Water						
**Template DNA 10 ng. (max of ~100 ng) Do not add. Only for calculation.						
Final Total Volume	5 µL	5 µL	10 µL	10 µL	20 µL	20 L
*Volume of nuclease free water will be determined by the volume of template DNA or the method of DNA delivery method. Add sufficient nuclease free water to arrive at the final total volume of the reaction.						
**Determine the DNA delivery method, if using wet delivery method then the maximum volume can be used substituting the addition of sterile water. For dry DNA delivery method the volume will be zero.						

C. Plate Setup

1. Dispense appropriate volume of reaction premix to each well.
2. Add template DNA if using the wet DNA delivery method.
3. Seal the plate with optical adhesive film.
4. Gently tap the plate(s) to mix the contents.
5. Centrifuge plate(s) to ensure elimination of bubbles and reaction contents accumulation at the bottom of well.

D. PCR Cycling

1. Place the plate(s) in the real time PCR thermal cycler and start the appropriate programmed file for thermal cycling.
2. Consult instrument manufacturer manual for cycling features and fluorophore optical detection properties.
3. Consult instrument manual for viewing and retrieving result files.
4. Given below is a suggested thermal cycling profile used successfully at Gene Link.

GeneAssays™ Real Time qPCR TaqMan® Thermal Cycling Profile			
Description	**Temperature**	**Time**	**Cycle(s)**
Taq Hot start Activation	95°C	10 minutes	1 cycle
Denaturation	95°C	15 seconds	40 Cycles
Annealing & Extension	60°C	1 minute	
If required a hold cycle at 12°C for infinity can be inserted at the end to retrieve amplified product.			

GeneAssays™ Real Time qPCR Molecular Beacon® Thermal Cycling Profile			
Description	**Temperature**	**Time**	**Cycle(s)**
Taq Hot start Activation	95°C	10 minutes	1 cycle
Denaturation	95°C	15 seconds	40 Cycles
Annealing-OPTICS ON	60°C	30 seconds	
Extension-OPTICS OFF	72°C	30 seconds	
If required a hold cycle at 12°C for infinity can be inserted at the end to retrieve amplified product.			

Appendix

Real Time Thermal Cyclers and Passive Dye Requirements

GeneAssays™ Genotyping PCR 2X Master Mix Without Reference Dye (Catalog No.: 41-1031-XX)
For use with the following real time thermal cyclers that do not require passive reference dye. The list is not comprehensive; please refer to instrument manufacturer for passive dye requirements and specifications
- Bio-Rad. CFX, iQ™, and Opticon™ Real-Time PCR Detection Systems
- Roche. LightCycler® Real-Time PCR Systems.
- All thermal cyclers for standard hot start PCR.

GeneAssays™ Genotyping PCR 2X Master Mix With ROX Reference Dye (Catalog No.: 41-1036-XX)
For use with the following real time thermal cyclers that requires passive reference dye. The list is not comprehensive; please refer to instrument manufacturer for passive dye requirements and specifications
-Thermo Fisher. 7500, 7500HT, 7300, QuantStudio™, StepOne™ and StepOnePlus™
- Agilent. Mx3005P™ and Mx400P™.

Preparation of Primer & Probe Assay Mix

Gene Link validated and optimized primer & assay mix concentration is given in the table below. This is a good starting point and can be optimized individually for custom primers and probes.

Gene Expression & Genotyping Assay		
Final 1X concentration		
	Gene Expression Concentration	**SNP Genotyping Concentration**
Each Primer	500 -900 nM	500 -900 nM
Probe(s)	250 nM	200 nM

Preparation of Primer & Probe Stock Solution

Gene Link supplies custom primers and probes as lyophilized solid or if requested as reconstituted solution. We suggest to prepare a stock solution of 100 μM of individual primers and probes in low TE (10 mM Tris pH8.0, 0.1 mM EDTA). Further dilution of these can be made to prepare the final 10X desired primer/probe(s) assay mix.

Reconstitution, Use & Stability of Lyophilized Fluorescent Probes & Oligos

All Gene Link custom oligo products including, molecular probes, RNA and siRNA includes a datasheet that contains the exact nmols, µg, or A_{260} units (OD Units) and other physical data. This data is important for reconstituting the product. All fluorescent probes are shipped in amber tubes to prevent exposure to light and minimize photo-bleaching. Gene Link guarantees the stability of oligos for 1 year and fluorescent molecular probes for 6 months if reconstituted and stored appropriately as detailed below.

In our experience unmodified oligos are stable for numerous years if reconstituted and stored properly. Avoid multiple freeze thaws; do not exceed 6-10 freeze thaw cycles. If the same oligo is intended to be used repeatedly then it is prudent to make a numerous aliquots of the stock solution and frozen.

Reconstitution & Storage

Gene Link fluorescent probes are supplied lyophilized in amber tubes to protect from light and to reduce photo bleaching. These are stable at room temperature for an extended period of time but should preferably be frozen upon receipt. TE buffer is recommended for dissolving the probes and oligonucleotides; EDTA inhibits the activity of the nucleases.

Preferred TE Buffer Reconstitution & Storage pH for Fluorescent Probes	
6-FAM, HEX, TET, ROX, and TAMRA	TE Buffer pH 7.5 or 8.0
Cy3, Cy3.5, Cy5, and Cy5.5	TE Buffer pH 7.0 or 7.5
Alexa dyes	TE Buffer pH 7.5 or 8.0
Cy dyes rapidly degrade in acidic pH	

Further dilution can be made in low TE buffer (10 mM Tris pH 8.0, 0.1 mM EDTA). After reconstitution store the stock solution at -80°C or -20°C. Fluorescently labeled oligos should be stored protected from light.

Preparation of Primer & Probe Stock Solution of 100 µM [100 pmols/µL]

Gene Link provides the exact amount of nmols of each probe supplied on the tube and on the Product Specifications Sheet included with the product. You can use this to reconstitute and prepare the stock solution.

Example: 6.6 nmols lyophilized product.
Dissolve the 6.6 nmol product in 66 µL of low TE pH 8.0 (10 mM Tris pH 8.0, 0.1 mM EDTA) to get a stock solution of 100 µM [100 pmols/µL]

Working Probe Stock Dilutions

The stock solution can be used for further dilution to prepare primer/probe assay mix as required.

Storage

For optimal long-term storage, it is recommended that the oligonucleotides should be stored dry at -20°C in the dark. If numerous experiments are planned using the same oligonucleotide, prepare aliquots, dry them and store the aliquots at -20°C.

Stability

Gene Link guarantees the stability of oligos for 1 year and fluorescent molecular probes for 6 months if reconstituted and stored appropriately as recommended by Gene Link. The stability can be increased several fold by instituting proper handling conditions, avoiding exposure to light and multiple freeze thaws.

Applied Biosystems Proprietary Dyes & Possible Substitutions		
Dye	**Absorbance max (nm)**	**Emission max (nm)**
VIC	538	554
HEX	535	556
NED	546	575
Cy3	550	570
PET	558	595
Cy3.5	588	604
ROX	575	602
Texas Red	583	603
LIZ	630	652
Cy5	646	667

Document Warranty and Liability

Information in this document is subject to change without notice. This document and all information presented in this document are written as a guide. Gene Link, Inc. does not warrant this document to be free of errors and assumes no responsibility for any errors that may appear in this document.
Gene Link disclaims all warranties with respect to this document, expressed or implied, including but not limited to those of merchantability or fitness for a particular purpose. In no event shall Gene Link be liable, whether in contract, tort, warranty, or under any statute or on any other basis for special, incidental, indirect, punitive, multiple or consequential damages in connection with or arising from this document, including but not limited to the use thereof.

Website

As the receipt of information on the Internet is highly dependent upon factors, including without limitations to, the user's computer, browser, operation system, etc., information may be perceived incorrectly. Therefore, Gene Link does not warrant or guarantee that the information contained on its website www.genelink.com is error free.

Product Warranty and Liability

Warranty: Gene Link makes no warranty of any kind, specifically disclaims and excludes all other warranties of any kind or nature, directly or indirectly, express or implied, including, without limitation, as to the suitability, productivity, durability, fitness for a particular purpose or use, merchantability, condition, or any other matter with respect to Gene Link products. Gene Link products are for research purposes only including custom products. There is no warranty or claim of its performance for any specific research application. All Gene Link products are guaranteed to meet or exceed the specifications stated. Each Gene Link product is shipped with documentation stating specifications and other technical information. If the product fails to meet the stated specifications the sole remedy is prompt replacement by Gene Link or within 30 days of purchase a refund of the purchased price.

Liability: Under no circumstances shall Gene Link be liable for any damages directly or indirectly related to Gene Link's products and services. Whether direct, incidental, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Gene Link products to perform in accordance with the stated specifications.

Research Use Only. Not for use in diagnostic or clinical procedures.

Notice to Purchaser: The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact support@genelink.com.

Gene Link, Inc.
190 Saw Mill River Road
Hawthorne, NY 10532
USA

Tel: (914) 769-1192
Email: support@genelink.com
www.genelink.com