PUREGENE
GENETIX BRAND

PRODUCT INSERT

GeneSure™ SYBR Green pPCR Master Mix (2x) Rox Solution Provided

Store at -20°C

Cat. No.	Concentration	Pack Size
PGK025A	2x	200 Reactions
PGK025B	2x	1000 Reactions

Features & Benefits

Simple and reproducible: A choice of optimized kits for different platforms, just add primers and template, reducing possible errors in set-up

Specific and sensitive: For detection of a wide range of template concentration, even from hard to obtain and low copy number samples

Fast protocols: Can be used with the new generation of fast cyclers without compromising performance

Instrument Compatibility

Specifically suitable for use on real-time instruments that measure ROX signal like ABI 7000, 7300, 7700, 7900, 7900HT and StepOne™. However the kit is also compatible with several instruments that do not require the use of ROX™, such as the Qiagen Rotor-Gene™ 6000, the Bio-Rad CFX96 or the Roche LightCycler® 480.

Description

The GeneSure™ SYBR Green Kit is a high-performance reagent designed for superior sensitivity and specificity on various real-time instruments. The GeneSure™ SYBR Green Kit employs a hot-start DNA polymerase, for high PCR specificity and sensitivity. GeneSureMix ™ is inactivate and possesses no polymerase activity during the reaction set-up, preventing non-specific amplification including primer-dimer formation.

For ease-of-use and added convenience, GeneSure™ SYBR Green is provided as a 2x mastermix containing all the components necessary for real-time PCR, including the SYBR® Green I dye, dNTPs, stabilizers ROX solution is provided separately.

Components	PGK025A	PGK025B
Reagent	**200 x 25µl Rxn**	**1000 x 25µl Rxn**
GeneSure™ SYBR Green Reaction mix (2x)	2 x 1.25ml	10 x 1.25ml
ROX Solution 50µM	50µl	250µl
Water, Nuclease Free	2 x 1.25ml	10 x 1.25ml

Shipping Conditions:
On Dry Ice or Blue Ice

Storage and Stability:
Store at -20°C and for longer use store at -70°C. Excessive freeze/thawing is not recommended. Since SYBR® Green I is light -sensitive, it is important to avoid prolonged exposure to light. When stored under optimum conditions, the reagents are stable for a minimum of 6 months from date of purchase.

Quality Control:
The GeneSure™ SYBR Green qPCR Kit and its components are extensively tested for activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination prior to release.

Safety Precautions:
Harmful if swallowed. Irritating to eyes, respiratory system and skin.

Notes:
1. GeneSure™ is a Trademark of Genetix.
2. This product insert is a declaration of analysis at the time of manufacture.
3. Research Use Only.

Reaction Protocol

Reaction mix composition:

Prepare a PCR master mix. The volumes given below are based on a standard 25µl final reaction mix and can be scaled accordingly.

ROX passive reference dye is supplied in a separate tube at 50 µM concentration. It can be added to a whole 2X master mix tube or to individual reaction mixture. ROX allows for correction of well-to-well variation due to pipetting inaccuracies and fluorescence fluctuations.

Table 1. Recommended amounts of ROX for a specific instrument.

Instrument	Amount of ROX per 25 µl reaction	Amount of ROX per 1.25 ml of	Final ROX
Applied Biosystems:7300, 7900HT, StepOne™, StepOnePlus™, ABI PRISM®7000 and 7700	0.05µl	5 µl	100nM
Applied Biosystems:7500 **Stratagene:**Mx3000P™, Mx3005P™, Mx4000 ®	0.05 µl 10X diluted*	5µl 10X diluted*	10 nM
Bio-Rad:iCycler ® iQ, iQ5 and MyiQ™, Opticon®, CFX96, CFX384 **Roche:**LightCycler ® 480, LightCycler® 2.0 **Corbett:**Rotor-Gene™ 3000, 6000 **Eppendorf:**MasterCycler™ ep *realplex* **Cepheid:**Smart Cycler	Not requied	Not required	Not requied

*Add 2µl of ROX Solution to 18 µl of Water, nuclease-free, mix and use 0.05 µl for 25 µl qPCR reaction.

Suggested thermal cycling conditions:

The PCR conditions described below are suitable for GeneSure™ SYBR Green Kit for the majority of amplicons and real-time PCR instruments. However, the cycling conditions can be varied to suit customer or machine-specific protocols. The critical step of the PCR is the 10 minute initial activation at 95°C. The detection channel on the real-time instrument should be set to (SYBR) Green or FAM.

Reagent	Volume	Final concentration
2x GeneSure™ SYBR Green	12.5µl	1x
Forward Primer*	variable	0.3µm
Reverse Primer	variable	0.3µm
Template	variable	
H_2O	Up to 25µl	-
Final volume	**25µl**	

*Final Primer Conc of 0.3 µM is optional in most cases, but may be individually optimized in a range of 0.05µM to 0.9µM

Cycles	Temperature	Time	Notes
1x	*95°C	*10min	Polymerase activation
40x	95°C	15s	
	55-60°C	30s	Temp. depends on the Tm of Primers
	72°C	30s	Acquire at end of step

***Non-variable parameter**

Optional analysis:

After the reaction has reached completion refer to the instrument instructions for the option of melt-profile analysis.

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General considerations

To help prevent any carry-over DNA contamination we recommend that separate areas be maintained for PCR set-up, PCR amplification and any post-PCR gel analysis. It is essential that any amplified PCR product should not be opened in the PCR set-up area.

Primers: The sequence and concentration of primer as well as the amplicon length can be critical for specific amplification, yield and overall efficiency of any real-time PCR. We strongly recommend taking the following into consideration when designing and running your PCR reaction:

- Use primer-design software, such as Primer3 or visual OMPTM (http://frodo.wi.mit.edu/primer3/ and DNA Software, Inc ; http://dnasoftware.com/ respectively). Primers should have a melting temperature (Tm) of approximately 60°C
- Optimal amplicon length should be 50-150bp
- A final primer concentration of 250nM is suitable for most PCR conditions, however to determine the optimal concentration we recommend a primer titration in the range of 0.1–1µM
- Use equimolar primer concentrations
- When amplifying from cDNA use gene-specific primers. If possible use intron-spanning primers to avoid amplification from genomic DNA

Template: it is important that the DNA template is suitable for use in PCR in terms of purity and concentration. Also, the template needs to be devoid of any contaminating PCR inhibitors (e.g. EDTA). The recommended amount of template for PCR is dependent upon the type of DNA used. The following should be considered when using genomic DNA and cDNA templates:

- **Genomic DNA:** use up to 1µg of complex (e.g. Eukaryotic) genomic DNA in a single PCR. We recommend using the Nucleopore® Genomic DNA Mini Kit for high yield and purity from both prokaryotic and eukaryotic sources
- **cDNA:** the optimal amount of cDNA to use in a single PCR is dependent upon the copy number of the target gene. We suggest using 100ng cDNA per reaction, however it may be necessary to vary this amount. To perform a two-step RT-PCR, we recommend using the Puregene® GENESCRIPT™ First Strand cDNA Synthesis Kit for reverse transcription of the purified RNA. For high yield and purity of RNA, use RNASure® Mini Kit (Nucleopore®)

$MgCl_2$: The $MgCl_2$ concentration in the 1x reaction mix is 3mM. In the majority of qPCR conditions this is optimal for both the reverse transcriptase and the hot-start DNA polymerase. If necessary, we suggest titrating the $MgCl_2$ to a maximum of 5mM.

PCR controls: It is important to detect the presence of contaminating DNA that may affect the reliability of the data. Always include a no template control (NTC), replacing the template with PCR-grade water. When performing a two-step RTPCR, set-up a no RT control as the NTC for the PCR.

Troubleshooting guide

Possible Cause	Recommendation
Problem No 1: No amplification trace AND No product on agarose gel	
Possible Cause	**Recommendation**
Error in protocol setup	Verify that correct reagent concentrations, volumes, dilutions and storage conditions have been used
Suboptimal primer design	Use primer design software or validated primers. Test primers on a control template
Incorrect concentration of primers	Use primer concentration between 100nM and 1µM
Template degraded	Re-isolate your template from the sample material or use freshly prepared template dilution
Primers degraded	Use newly synthesized primers
Template contaminated with PCR inhibitors	Further dilute template before PCR or purify template and resuspend it in PCR-grade H2O
Template concentration too low	Increase concentration used
Cycling conditions not optimal	Increase extension/annealing times, increase cycle number, reduce annealing temperature
Problem No 2: No amplification trace AND Product on agarose gel	
Error in instrument setup	Check that the acquisition settings are correct during cycling
Problem No 3: Non-specific Amplification product AND Primer-dimers	
Suboptimal primer design	Redesign primers using appropriate software or use validated primers
Primer concentration too high	Test dilution series of primer concentrations until primer dimer/non-specific amplification products disappear
Primer concentration too low	Titrate primers in the concentration range of 100nM - 1µM
Primer annealing temperature too low	Increase PCR annealing temperature in increments of 2°C until primer dimer/non-specific amplification products disappear
Template concentration too low	Increase template concentration
Template concentration too high	Reduce template concentration until non-specific products disappear
Extension time too long	Reduce extension time to determine whether non-specific products are reduced
Problem No 4: Late Amplification trace	
Activation time too short	Ensure the reaction is activated for 10min at 95°C before cycling
Annealing temperature too high	Decrease annealing temperature in steps of 2°C
Extension time too short	Double extension time to determine whether the cycle threshold (CT) is affected
Template concentration too low	Increase concentration if possible
Template with high secondary structure	Increase reverse transcription reaction time up to 30min: Increase reverse transcription reaction temperature up to 45°C
Template is degraded	Re-isolate template from sample material or use freshly prepared template dilution
Suboptimal design of primers	Redesign primers using appropriate software or use validated primers
Primer concentration too low	Increase concentration of primer in 100nM increments
Problem No 5: PCR efficiency below 90%	
Extension time is too short	Increase extension time
Primer concentration too low	Increase concentration of primer in 100nM increments
Suboptimal design of primers	Redesign primers using appropriate software or use validated primers
Problem No 6: PCR efficiency above 110%	
Template is degraded or contains PCR inhibitors	Re-isolate template from sample material or use freshly prepared template dilution or purify template and resuspend it in H2O
Non specific amplification and/or primer dimers	Use melt analysis and 4% agarose gel electrophoresis to confirm presence of non-specific amplification products.See above for preventing/removing non-specific products

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