GENDX

NGSgo®-AmpX

Instructions For Use

Edition 2, 2019/08

Amplification for downstream sequencing applications

MICA, MICB

For Research Use Only

MAT 2341300

www.GenDx.com

IMPORTANT NOTES AND UPDATES

Updates Edition 2

- Section ‘Ordering information’ now includes both the company name ‘Genome Diagnostics B.V.’ and the trade name ‘GenDx’.
- Appendix A. Contamination control now states more clearly that pre-amplification and post-amplification areas should be separated.

CONTENT

Disclaimer

GenDx has made every effort to ensure that this IFU is accurate. Information in this IFU is subject to change without notice. GenDx reserves the right to make improvements to this IFU and/or to the products described in this IFU at any time without notice.

If you find information in this manual that is incorrect, misleading, or incomplete, we would appreciate your comments and suggestions. Please send them to info@gendx.com.

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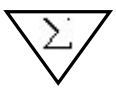
1 KEY TO SYMBOLS

MAT	Material number
COMP	Components
LOT	Batch code / Lot number
REF	Catalogue number
	Consult Instructions For Use
	Contains reagents for N tests
	Legal manufacturer
Store at -20°C	Store at -20°C
	Add liquid
	Use-by date

2 KIT CONTENT

For full description of kit content see kit content label on the box or visit our website (www.gendx.com).

3 SHIPPING AND STORAGE

NGSgo®-AmpX kits are:

- Shipped at -20°C and should be stored at -20°C upon arrival.
- Stable until the kit expiration date, indicated on the box label, when stored at -20°C.
- After dissolving primers in nuclease-free H_2O, primers are stable for 12 months when stored at -20°C.

4 TECHNICAL ASSISTANCE

For technical assistance and more information:

Email: support@gendx.com
Website: www.gendx.com
Phone: +31 30 252 3799

Or contact your local GenDx distributor, www.gendx.com.

5 WARNING AND PRECAUTIONS

Product Use Limitations

NGSgo®-AmpX MICA, MICB is for Research Use Only (RUO) and not to be used in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

- To ensure the best performance, use the NGSgo®-AmpX kits with the materials, reagents and equipment recommended in section 8 “Equipment and reagents to be supplied by user”. Use of materials other than specified, must be validated by the user.
- Reconstitution or dilution of primers in volumes other than described in this IFU can lead to incorrect results and is therefore strongly discouraged.

- GenDx cannot provide support for any problems resulting from non-adherence to this Instructions for Use document.
- Please take special note of section 11 “Contamination control”.

Safety Information

- When working with chemicals always wear a suitable lab coat, disposable gloves, and protective goggles. For more information please consult the appropriate material safety data sheets (MSDSs) available from the product supplier.

6 PRINCIPLE

NGSgo®-AmpX MICA, MICB consists of dedicated primer sets for amplification of Major Histocompatibility Complex Class I chain-related protein A and B genes.

7 PROCEDURE

Locus-specific amplification is performed in a thermal cycler using the amplification primer mix, template genomic DNA and GenDx-LongMix. The resulting locus-specific amplicon(s) can subsequently be used for identification of both alleles by means of Next-Generation Sequencing (NGS) technologies.
It is possible to pool MICA and MICB amplicons before starting the NGSgo workflow. MICA and MICB amplicons can also be pooled with HLA amplicons generated with other NGSgo-AmpX primers.

8 EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

- Ice
- Pipettes and pipette tips with hydrophobic filters
- Thermal cycler
- Microcentrifuge
- Vortex
- PCR tubes (use thin-walled 0.2 ml PCR tubes recommended by the manufacturer of your thermal cycler)
- Agarose gel electrophoresis system

9 IMPORTANT NOTES BEFORE STARTING

Sample Preparation

- Purified DNA should have an A_{260}/A_{280} ratio of ~1.8.
- If necessary, DNA should be diluted in nuclease-free H_2O before use.
- The optimal amount of template DNA to use in the reaction is 40 ng. However, template DNA in the range of 20 - 80 ng (in 1 - 2 µl) can be used without affecting results.
- To streamline the process, validate your DNA purification procedure so you can use a set volume corresponding to 20 - 80 ng DNA.
- Blood samples should be collected in ACD or EDTA anticoagulated tubes. Do NOT use heparinized samples. Heparin has an inhibitory effect on a PCR.

Assay Set-up

- **Set up all reactions on ice**.
- Prepare a volume of reaction mix at least 10% greater than required for the total number of assays to be performed.

NGSgo®-AmpX Primer Preparation

1. Centrifuge all tubes for at least one minute before opening for the first time to ensure that the orange pellet is at the bottom of the tube.

2. Resuspend each primer (96 reaction tube) in 106 µl nuclease-free H_2O (provided).

3. Invert the tubes a couple of times, thoroughly vortex each tube and centrifuge the tubes for one minute.

4. Repeat step 3 at least two times.

10 PROTOCOLS

PROTOCOL 1: AMPLIFICATION PROCEDURE

1. Set up all reactions on ice.

2. Prepare separate reaction mixes for MICA and MICB.

3. Thaw GenDx-LongMix, nuclease-free H_2O, and primer solutions. Mix the solutions thoroughly and centrifuge briefly before use.

4. Prepare a reaction mix as shown in Table 1.

It is important to include at least one negative control in every PCR setup lacking template nucleic acid in order to detect possible contamination.

Table 1: Composition of reaction mix for locus-specific amplification.

Component	
Nuclease-free H_2O	4.5 - 5.5 µl
GenDx-LongMix (4x)	2.5 µl
AmpX primer (red cap)	1 µl
Template DNA (~40 ng/reaction)	1 - 2 µl
Total Volume	10 µl

5. Mix the reaction mix thoroughly and centrifuge briefly.

6. Dispense the reaction mix into each PCR tube/well.

7. Add 1 - 2 µl template DNA (20 - 80 ng) or H_2O as a negative control to each tube containing the reaction mix.

8. Program the thermal cycler according to the manufacturer's instructions using the conditions outlined in Table 2.

9. Important: Use an artificial hot start to ensure PCR specificity: place the tubes immediately into a thermal cycler that is heated to 95°C and start the cycling program as outlined in Table 2.

Table 2. Cycling protocol for amplification.

Step	Temp	Time
Initial denaturation	95 °C	3 min
3-step cycling		
Denaturation	95 °C	15 sec
Annealing	65 °C	30 sec
Elongation	68 °C	6 min
35 cycles		
Final elongation	68 °C	10 min
Cooling	15 °C	∞

10. After amplification samples can be stored at 2 - 8°C.

11. Confirm that amplicons are generated by agarose gel electrophoresis. Prepare a 1% w/v agarose gel, and analyze 2 µl of each PCR assay. The size of the amplicons should be ~13 kb.

11 APPENDIX A. CONTAMINATION CONTROL

IMPORTANT:

It is extremely important to include at least one negative control in every PCR setup that lacks template nucleic acid to detect possible contamination.

General Physical Precautions

- Please note that opening tubes after PCR can release amplification products by means of aerosols that can contaminate your working area. For this reason the working areas for pre-amplification and post-amplification procedures must be separated, as described in EFI and ASHI Standards.
- Ideally, pre-amplification and post-amplification procedures should be performed in separate rooms.
- Resuspension of primers and preparation of the locus-specific amplification reaction mix should be performed in the pre-amplification area. Thermal cycling and all protocols thereafter should be performed in the post-amplification area.
- Use a separate set of pipettes for the pre-amplification procedures. Use of pipette tips with hydrophobic filters is strongly recommended.

- In case of contamination, laboratory benches, apparatus, and pipettes need to be decontaminated, for example with a 1% Trigene disinfectant according to the manufacturer's instructions. Preferably, contaminated samples and reagents are discarded.
- Prepare and freeze small aliquots of primer solutions. Use of fresh nuclease-free H_2O as provided in the kit is strongly recommended.

General Chemical Precautions

- PCR stock solutions can also be decontaminated using UV light. This method is laborious however, and its efficiency is difficult to control and cannot be guaranteed. We recommend storing solutions in small aliquots and using fresh aliquots for each PCR.
- Another approach to prevent amplification of contaminating DNA is to treat individual reaction mixtures with DNAse or restriction enzymes that cut between the binding sites of the amplification primers used, before adding the template DNA sample.

12 TROUBLESHOOTING GUIDE

Little or no PCR product	
Sample does not amplify for MICA.	A deletion of the entire MICA gene has been described and is relatively common in some populations, such as Japanese, Koreans, and Angaite Amerindians.
GenDx-LongMix was not added to the amplification mix or not mixed properly when added.	Repeat amplification paying attention to the addition and mixing of GenDx-LongMix with the amplification mix.
Cycling conditions not optimal.	When using a fast thermal cycler, reduce the ramp rate to 1°C/s.
DNA concentration not optimal.	Re-quantify the DNA and adjust to at least 20 ng/µl (40 ng/µl is optimal). If the sample concentration is below the recommended range and little or no amplification product is visible, sequence the sample anyway. Acceptable sequence and typing may still be achievable.
Poor quality or degraded genomic DNA.	Run genomic DNA on a 1% agarose gel to evaluate quality. Purified DNA should have an A260/A280 ratio ~1.8.

13 LIMITED LICENSE AGREEMENT

Use of this product signifies the agreement of any purchaser or user of the GenDx NGSgo®-AmpX kits to the following terms:

- The NGSgo®-AmpX kits may be used solely in accordance with the NGSgo®-AmpX Instructions For Use document. GenDx grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the GenDx NGSgo®-AmpX Instructions For Use document and additional protocols available at www.GenDx.com.

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- For updated license terms, see www.GenDx.com.

Trademarks: NGSgo® is a registered trade mark of Genome Diagnostics.

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ORDERING INFORMATION

GenDx products are supported either directly or by your local GenDx distributor or reseller. Please contact your local GenDx distributor (www.GenDx.com), or GenDx Customer Support team at +31 302 523 799 or **order@gendx.com** for any product information or quote request.

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