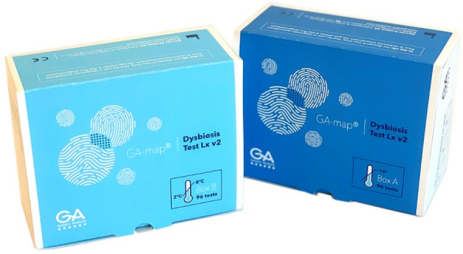

S8-044-A1 GA-map Dysbiosis Test Installation Guide

Genetic Analysis

GA-map® | Dysbiosis Test Lx v2

INSTALLATION GUIDE
for GA-map® Dysbiosis Test Lx v2

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OVERVIEW

This guide provides the information required to prepare a laboratory for performing the GA-map® Dysbiosis Test Lx v2 assay. Requirements for instruments, materials, and reagents are described.

INSTALLATION CHECK LIST

The following installation checklist summarizes the elements that must be completed during the installation phase. Mark each item as it is completed. Once these prerequisites have been met, please contact Genetic Analysis AS to schedule the training.

- Instruments and consumable items for Step 1 Genomic DNA extraction (see tables on page 4-6) must be acquired
- Instruments and consumable items for Step 2 Amplification of the bacterial 16S rRNA gene – Step 6 Hybridization and Signal Detection (see tables on page 6-8) must be acquired
- All instruments, including the Luminex detection system, must be installed and ready for use
- Operators must have been trained in use and maintenance of the Luminex detection system

RECOMMENDED SAFETY EQUIPMENT

 Fecal samples should be treated as potentially infectious material until inactivation and require the use of BSL-2 grade laboratory equipment and precautions. This involves the use of appropriate PPE, biological safety cabinet, proper waste disposal and risk-minimizing routines for sample handling. Appropriate skin and eye protection should be worn during use of the DNA isolation kit (mag™ maxi, LGC genomics). For all other steps of the assay, suitable lab coat and disposable gloves should be used.

COMPUTER REQUIREMENTS

The GA-map® Analyzer is a cloud-based software compatible with most web browsers. See the GA-map® Analyzer Manual for details.

LABORATORY REQUIREMENTS

The GA-map® Dysbiosis Test Lx v2 requires a typical molecular laboratory set-up. It is recommended that the laboratory contains, at a minimum, dedicated pre-PCR, template-free, and PCR/post-PCR zones. The zones should be separated to prevent contamination.
The table below contains an overview of the different laboratory procedures that are performed in the individual zones, in addition to instruments and equipment needed in the different workstations. Please refer to the Equipment, materials, and reagents required section for equipment specifications. To equip the lab with appropriate pipettes, please refer to the IFU for volumes to be pipetted.

Zone	Lab procedures	Workstation
Pre-PCR	Sample preparation and gDNA extraction. Addition of template for the amplification step.	Biological safety cabinet equipped with: • Vortex mixer • Decapper for Lysing Matrix-E tubes • Appropriate single- and 8-channel pipettes w/ wide orifice tips Open bench equipped with: • Bead beater • Plate centrifuge for Lysing Matrix-E tubes • Water bath • DNA extraction robot • Vortex mixer • Microcentrifuge • Dispenser pipette w/ tips • Single- and 8-channel pipettes with tips • Ice or cooling elements for other reagents and plate
Template- free	Mastermix preparation for the amplification, clean-up, End-Labeling, and Hybridization steps.	PCR cabinet with available equipment: • Vortex mixer • Microcentrifuge • Dispenser pipette w/ tips • Single channel pipettes w/ tips • Freezing block for enzymes • Ice or cooling elements for reagents and plate
PCR/Post-PCR	Amplification reaction. The quantification step. Addition of template for the End-labeling step and the End-labeling reaction. The Hybridization and signal detection step.	Open bench equipped with: • Plate centrifuge for quick spin • Thermal cycler • DNA quantification system • Ring magnet plate • Luminex detection platform • Vortex mixer • Microcentrifuge • Dispenser pipette w/ tips • Single- and 8-channel pipettes w/ tips • Ice or cooling elements for reagents and plate • Freezing block for plates

EQUIPMENT, MATERIALS, AND REAGENTS REQUIRED

The lists below describe the required equipment, materials, and reagents for the GA-map® Dysbiosis Test Lx v2. All items are required, unless otherwise specified. Genetic Analysis must be informed of any deviations from these requirements and a separate validation might be necessary.

The quantity listed for all equipment is based on the set-up described in the Laboratory Requirements section above and describes the minimum recommended quantity. It should be noted that different laboratory set-ups (e.g. with additional zones) might affect the recommended quantity.

FOR STEP 1 – GENOMIC DNA EXTRACTION

List of equipment

	Equipment type	Specifications/ requirement	Alternative or recommended option	Quantity
	Decapper for Lysing Matrix-E tubes	Capacity: 8-channel capper/decapper for matrix tubes	8-Channel Screw Cap Decapper (4105MAT), Thermo Scientific	1
			AlteCap™ Switch (404000) with Cassette 12/96 – Internal thread Matrix screw caps (404014), AltemisLab	
	Bead beater	FastPrep-96™ Homogenizer w/96-well plate insert (116010500), MP Biomedicals	No validated alternatives	1
	Plate centrifuge for Lysing Matrix-E tubes	Capacity: Lysing Matrix-E 96 well rack with tubes (6 cm height) Force: 1300 rcf	Eppendorf™ Centrifuge Centrifuge 5804/5810 w/ rotor for deepwell plates, Eppendorf	1
	Water bath	Capacity: deep well 96 plate Temperature: 65°C	Any	1
	DNA extraction robot	KingFisher Flex – 96 deep well head (540 0630) w/magnetic micro-plate Separator, installed with KF Flex 96 KF heating block (2407 5420), Thermo Scientific	No validated alternatives	1
	Vortex mixer	Speed: ~2800 rpm	Any	2
	Micro Centrifuge	Capacity: 1.5ml/ 2ml tubes (spin down only)	Any	1
	Dispenser pipette w/tips	Volumes to be dispensed: 20, 200, 250, 270 and 720 µl	Multipette®E3/E3x (4987000371/4987000380), Eppendorf	1
	Pipettes single channel w/tips	Volumes: see IFU Wide orifice tips recommended for pipetting of fecal samples prior to gDNA extraction	Any	Depends on volume range
	Pipettes 8- channel w/tips	Volumes: see IFU Wide orifice tips recommended for pipetting of fecal samples prior to gDNA extraction	Any	Depends on volume range
	Ice or cooling blocks	For keeping reagents/sample intermediates cold during handling	Any	NA

List of materials

	Material	Specifications/ requirement	Alternative or recommended option
	Lysing Matrix-E tubes	Lysing Matrix E, 96-tube rack, barcoded tubes, 1 Rack (116984001B), (note: individual Matrix tubes with screw cap), MP Biomedicals	No validated alternatives
	Tube for Lysis mix	Volume: 5, 15 or 50 ml	Any
	Deep well plate for DNA extraction robot	KingFisher Deepwell 96 Plate, V- bottom, (95040450), Thermo Scientific	96 Well Plate SW 2ml with V- Bottom PP (BAKR43001-0504), VWR
	Deep well Tip Combs for DNA extraction robot	KingFisher 96 tip comb for DW magnets, (97002534), Thermo Scientific	96 Tip Comb PP (BAKR43001-0505), VWR
	Elution plate for DNA extraction robot	KingFisher 96 KF microplate (200µL), (97002540), Thermo Scientific	Well Plate 96 SW 0.2ml with V- Bottom PP (BAKR43001-0506), VWR
	Adhesive PCR plate seal	Suitable for deep well plate for DNA extraction robot	Adhesive PCR Plate Seals (AB0558), Thermo Scientific
	Microtiter sealing tape	Suitable for plates for DNA extraction robot	Adhesive Plate Seals (AB0580), Thermo Scientific
	Microtiter plate w/seal for gDNA dilution	Capacity: 96-well, ≥250µl	Any
	Reagent reservoir	≥25 ml	Any

List of reagents

	Reagent	Specifications/ requirement	Alternative or recommended option
	Extraction control positive (optional)	Fecal sample of known quality	Any
	DNA extraction reagent kit	mag™ maxi DNA purification kit, 288 tests (NAP40430), LGC Genomics **Note!** Protease must be stored at -20°C upon reception	No validated alternatives
	Ethanol (for extraction kit)	96-100% ethanol ≥500ml	Any
	Acetone (for extraction kit)	≥99% acetone ≥350ml	Any
	Water for dilution of gDNA and as PCR ctrl neg.	Sterile, Nuclease-free water ≥500ml	Any

FOR STEP 2 – AMPLIFICATION OF THE BACTERIAL 16S RRNA GENE TO STEP 6 –HYBRIDIZATION AND SIGNAL DETECTION
List of equipment

	Equipment type	Specifications/ requirement	Alternative or recommended option	Used in assay steps	Quantity
	Plate centrifuge	Capacity: 96-well plate (quick spin down only)	Any	Step 2 Step 3 Step 4 Step 5 Step 6	1
	Thermal cycler	VeritiPro™ 96-Well Thermal Cycler (A48141), Applied Biosystems	Veriti™ 96-Well Thermal Cycler (4375786), Applied Biosystems T100™ Thermal Cycler (186- 1096), Bio-Rad	Step 2 Step 4 Step 5 Step 6	1
	DNA quantification system	FLUOstar OMEGA Microplate Reader with filter-based absorbance (415-103) w/ appropriate filters for the quantification assay, BMG LabTech	Qubit® Fluorometer 3.0 (Q33216) or 4.0 (Q33238) or Flex (Q33327), Invitrogen	Step 3	1
	Ring Magnet Plate	Capacity: 96-well Hybridization plate, 100 µL	96-Well Ring Magnet Plate (S380), Permagen	Step 6	1
	Luminex detection platform	Luminex® 200™ system with xPONENT® version 4.2 or higher (LX200- XPON-IVD/RUO), Luminex*	MAGPIX® system or NxTAG®-Enabled MAGPIX® system with xPONENT® version 4.2 or higher (MAGPIX-XPON4.1-CEIVD), Luminex*	Step 6	1
	Vortex mixer	Speed: ~2800 rpm	Any	Step 2 Step 3 Step 4 Step 5 Step 6	3 (including one of the two listed in Step 1)
	Micro Centrifuge	Capacity: 2 ml tubes (spin down only)	Any	Step 2 Step 3 Step 4 Step 5 Step 6	3 (including the one listed in Step 1)
	Dispenser pipette w/tips	Volumes to be dispensed: 20 µl and 40 µl	Multipette®E3/E3x (4987000371/4987000380), Eppendorf	Step 2 Step 5 Step 6	2
	Pipettes single channel w/tips	Volumes: see IFU	Any	Step 2 Step 3 Step 4 Step 5 Step 6	Depends on volume range

	Pipettes 8- channel w/tips	Volumes: see IFU	Any	Step 2 Step 3 Step 4 Step 5 Step 6	Depends on volume range
	Ice or cooling blocks	For keeping reagents/sample intermediates cold during handling	Any	Step 2 Step 3 Step 4 Step 5 Step 6	NA
	Freezing block for tubes	Capacity: 1.5/ 2 ml reagent tubes For use during handling of enzymes	Benchtop cooler (5115- 0012), Thermo Scientific	Step 2 Step 4 Step 5	1
	Freezing block for plates	Capacity: 96-well microtiter plate For use during End- labeling	PCR-Cooler 0.2 mL (3881000031), Eppendorf	Step 5	1

*Additional items for use and maintenance are required: kits for calibration and performance verification, sheath fluid or drive fluid, and an ultrasonic bath (40-60 kHz)

List of materials

	Material	Specifications/ requirement	Alternative or recommended option	Used in assay steps
	Microcentrifuge tubes for mixing of reagents	Volume: 1.5, 2 and 5 ml	Safe-Lock tubes, Eppendorf	Step 2 Step 4 Step 5 Step 6
	Microtiter plate for PCR/End- labeling	Capacity: 96-well PCR grade	96-Well Semi-skirted Flat Deck PCR Plates (AB1400), Thermo Scientific	Step 2 Step 5
	8-cap sealing strips	Suitable for microtiter plate	Flat PCR Caps, strips of 8 (AB0784), Thermo Scientific	Step 2 Step 5
	Microtiter sealing tape	Suitable for microtiter plate	Adhesive Plate Seals (AB0580), Thermo Scientific	Step 2 Step 5
	Tubes/plates for DNA quantification	Suitable for the selected DNA quantification system	For use with plate reader: Nunc™ 96-Well Microplate, Black (237108), Thermo Scientific	Step 3
			For use with Qubit® 3.0/4.0 Fluorometer: Qubit™ Assay Tubes (Q32856), Invitrogen	
			For use with Qubit® Flex Fluorometer: Qubit™ Flex Assay Tube Strips (Q33252), Invitrogen	
	Hybridization plate	Thermowell™ 96-Well Polycarbonate PCR Microplates, Model P (6509), Corning	96-well Twin.tec™ PCR Plates, Unskirted, Divisible (0030133358), Eppendorf	Step 6
	Sealing film for Hybridization plate	Microseal® 'A' (MSA5001), Bio-Rad	No validated alternatives	Step 6
	Pierceable foil for Hybridization plate**	Pierceable foil to cover 96-well plate	Any	Step 6
	Reagent reservoir	≥25 ml	Any	Step 4 Step 6

**Only applicable if using the NxTAG®-enabled MAGPIX® detection platform

List of reagents

	Reagent	Specifications/ requirement	Alternative or recommended option	Used in assay steps
	Water for PCR control neg.	Sterile, Nuclease-free water, same as used for dilution of gDNA	Any	Step 2
	Assay kit for DNA quantification	For use with plate reader: Quant-iT™ 1X dsDNA HS Assay (Q33232), ThermoFisher	Quant-iT™ PicoGreen™ dsDNA Assay Kit (P11496), Thermo Fisher	Step 3
		For use with Qubit® Fluorometer: Qubit™ 1X dsDNA HS assay kit (Q33231), ThermoFisher	Qubit™ dsDNA HS assay kit (Q32854), ThermoFisher	

TRAINING PLAN

Training will be scheduled following system set-up. Genetic Analysis will train users in performing the process of GA-map® Dysbiosis Test Lx v2 from fecal gDNA extraction (or from PCR, if the extraction method differs from that described in the IFU) to generation of the reports. Basic instrument operation and use of the software will be included in the training. Those being trained will be required to have a basic knowledge of Microsoft Windows and use of general laboratory equipment and tools.
Operators must have been trained in use and maintenance of the Luminex system prior to the tech transfer.

CONTACT INFO

We are happy to help you with your inquiries.
Technical Support: **support@genetic-analysis.com**
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Documents / Resources

	**GA S8-044-A1 GA-map Dysbiosis Test** [pdf] Installation Guide S8-044-A1 GA-map Dysbiosis Test, S8-044-A1, GA-map Dysbiosis Test, Dysbiosis Test, Test

References

- **Home - Genetic Analysis**
- **User Manual**