BIOSYNEX AMPLIQUICK® Fecal Pretreatment

Kit for the pretreatment of stool samples..

EN

Ref. 3150065

For professional *in vitro* diagnostic use only.

1 I INTENDED USE

BIOSYNEX AMPLIQUICK® Fecal Pretreatment is a manual kit for the pretreatment of fecal samples to screen for intestinal parasites through gene amplification. It is designed to release nucleic acids from the biological samples. It comes in the form of vials pre-filled with ready-to-use grinding beads and pretreatment buffer. The kit is intended to be used by laboratory staff for *in vitro* molecular diagnostics only.
The kit was developed and validated to be used exclusively with the following PCR kits:
BIOSYNEX AMPLIQUICK® Helminths (Ref. 3150066) for the qualitative detection by PCR of 10 helminth targets.
BIOSYNEX AMPLIQUICK® Protozoans (Ref. 3150067) for the qualitative detection by PCR of 10 protozoan targets.

2 I PRINCIPLE

Tests based on screening for nucleic acids of specific pathogens such as intestinal parasites help identify infected people. The BIOSYNEX AMPLIQUICK® Fecal Pretreatment kit optimises the release of DNA contained in the biological samples in order to perform molecular detection testing, which helps identify both silent infections in asymptomatic patients as well as acute infections in symptomatic patients.
The BIOSYNEX AMPLIQUICK® Fecal Pretreatment is an *in vitro* test based on the physico-chemical lysis of the cell membrane components or the wall of the parasites, their cystic forms or the eggs. The lysis stages are optimised to increase the ratio of non-human DNA extracted. The first stage involves mechanical grinding using garnet beads in the vial, which is put under agitation. The second stage of pretreatment involves heating to 95°C. The pretreatment medium is specifically designed to allow the DNA to separate from impurities, PCR inhibitors and enzymes likely to degrade the DNA and which are present in large quantities in faeces. After centrifugation, the DNA remains suspended in the supernatant, while the sample matrix is eliminated in the pellet. Nucleic acids can be extracted from the supernatant obtained using a suitable kit.

3 I KIT CONTENTS

Equipment provided
100 wooden spatulas
100 sampling spoons calibrated at 200 mg with levelling accessories
100 vials containing 1 g of grinding beads and 1 mL of pretreatment buffer
Instructions for use

Equipment required but not supplied
Stool sample containers
Powder-free disposable gloves
Micropipette & filter tips
Bead mill homogeniser for 2 mL vials (Bead Ruptor Elite, Omni)
Water bath or block thermostat at 95°C
Centrifuge for 2 mL vials (preferably RCF 11000 g)

4 I PRECAUTIONS

- For *in vitro* diagnostic use. For professional laboratory use only.
- Use the kit and its components by the expiry date only.
- For best results, carefully follow the storage procedure and conditions.
- In the event of damage to the packaging only (no breakage or leakage), the kit remains usable. If a vial is damaged, if it leaks or if it has arrived closed incorrectly, do not use it.
- Do not eat, drink or smoke while carrying out the sample pretreatment protocol.
- Follow good laboratory practice. Use powder-free disposable laboratory gloves throughout the procedure. Consider the samples potentially infectious and handle them with care, as per laboratory guidelines.
- If the sample or reagent spills or splashes, clean it up using a suitable disinfectant.
- Dispose of contaminated or empty kit components in a biohazard waste bin. Comply with local regulations on biowaste disposal.
- If using the BIOSYNEX AMPLIQUICK® Fecal Pretreatment kit leads to death or serious deterioration of health, the manufacturer and the local competent authority must be notified. If in doubt, report it.
- Safety data sheet available on request.

5 I STORAGE AND STABILITY

The kit is shipped at a temperature between 15°C and 25°C and the components must be stored at this temperature when they arrive. Under such conditions, the reagents remain stable until the expiry date specified.
The colour of the pretreatment buffer (orange) could change over time; this does not make it any less effective. If crystals appear or if a precipitate forms in the buffer, incubate the vials in question between 37°C and 70°C until the crystals or precipitate dissolves.

6 I SAMPLE COLLECTION AND STORAGE

- Collect the stool samples in suitable sampling containers without preservatives.
- Once a stool sample has been placed in the pretreatment medium, it will remain stable for 4h at room temperature, or for 48h if it is stored at 4°C, or at -20°C for longer storage.

Clinical samples must be transported in accordance with local regulations on the transport of infectious agents.

7 I TREATMENT PROTOCOL

1. Homogenise the stool using the wooden spatula provided.
2. Use the calibrated spoon (plunger “1” downwards) to collect a sample so as to fill up the end of the groove of the spoon.
3. Use the levelling accessory marked “2, 3” by holding it flat to remove any excess.
4. Empty the contents of the spoon into a vial containing beads and pretreatment buffer by pushing the plunger marked “1” forward.
5. If the stool is very loose, collect 200 µL using a micropipette and filter tip and transfer it into a vial containing beads and pretreatment buffer.
6. Using a pipette and filter tip, add 10 µL of the procedural control provided in either the BIOSYNEX AMPLIQUICK® Helminths or the BIOSYNEX AMPLIQUICK® Protozoans amplification kit.
7. Recap the vial and place it in the homogeniser in order to perform the mechanical grinding of the sample for a 1-minute and 10-second cycle on the Ruptor Elite Bead Mill by entering the following parameters: speed 4m/s, 2 cycles, duration 30 seconds, pause duration 10 seconds.
8. Incubate for 5 minutes at 95°C in the water bath or in a block thermostat.
9. Centrifuge the vial for 1 minute at 11000 g at room temperature (between 15°C and 25°C). Comment: If the centrifuge does not allow for this centrifugation speed, increase the centrifugation time proportionally (e.g. 2 minutes at 5500 g).
10. Collect the supernatant with a micropipette and a filter cone and transfer it to a clean, empty vial (nuclease free plastic).
11. Extract/purify the DNA before using PCR to amplify genes.

NB: BIOSYNEX AMPLIQUICK® Helminths (Ref. 3150066) and BIOSYNEX AMPLIQUICK® Protozoans (Ref. 3150067) amplification kits are not compatible with a DNA extract obtained using the Macherey-Nagel NucleoSpin DNA Stool kit.

8 I PERFORMANCES

This BIOSYNEX AMPLIQUICK® Fecal Pretreatment kit was developed and validated using the BIOSYNEX AMPLIQUICK® Helminths (Ref. 3150066) and BIOSYNEX AMPLIQUICK® Protozoans (Ref. 3150067) amplification kits. Please see the instructions for use for these products if you would like more information about their performance.

9 I INTERFERENCES

There is no known interference to report with the reagents of the BIOSYNEX AMPLIQUICK® Fecal Pretreatment kit.

10 ILIMITATIONS

You can use your own DNA extraction systems or commercial kits. We recommend respecting a 2:1 ratio of test volume to elution volume for the DNA extraction step (e.g. if 200µL of pretreatment supernatant is used for extraction, the DNA is eluted in 100µL).The calibrated spoons provided in the kit make it possible to collect the reference reproducible quantity for samples, namely 200 mg. However, the weight of the sample can fluctuate positively or negatively due to the consistency of the stool. A sample weight between 160 and 250 mg does not entail any significant differences in the performance of the BIOSYNEX AMPLIQUICK® Helminths and BIOSYNEX AMPLIQUICK® Protozoans amplification kits.

11 IBIBLIOGRAPHY

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SYMBOLS

	Consult instructions for use		Contains sufficient for <n> tests		Catalogue number
	*In vitro* diagnostic medical device		Temperature limit		Do not re-use
	Manufacturer		Batch code		Use-by date
	Date of manufacture		Do not use if package is damaged and consult instructions for use		Not for self-testing
	Not for near-patient testing		Unique Device Identifier		Authorized Representative in Switzerland
	Importer		Instructions for use		Vial with pretreatment buffer and grinding beads
	Wooden spatula		Calibrated sampling spoon		Levelling accessory

IFU_3150065_EN_V01202205R04
Date of last revision: 11/2022
Latest changes: Addition of precisions in the protocol and translation corrections