CE

IVD

For Professional Use Only

EPh

Detection agarose kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

EPh detection agarose kit is a reagent kit intended for electrophoretic detection of the amplified products in agarose gel.

2. PRINCIPLE AND PROCEDURE

EPh detection agarose kit is based on electrophoretic separation of amplified DNA fragments in agarose gel with following UV-detection.

3. CONTENT

EPh detection agarose kit is produced in 4 forms:

EPh detection agarose kit variant 200, **REF** K5-200-CE,

EPh detection agarose kit variant 300, **REF** K5-300-CE,

EPh detection agarose kit variant genotype-200, **REF** K6-200-CE

EPh detection agarose kit variant genotype-300, **REF** K6-300-CE

EPh detection agarose kit variants 200 or 300 include:

Reagent	***Description***	***Variant 200***		***Variant 300***	
		Volume (ml), Mass (g)	***Quantity***	***Volume (ml), Mass (g)***	***Quantity***
Tris-borate buffer (TBE) concentrated with ethidium bromide	orange clear liquid	50 ml	1 vial	75 ml	1 vial
Agarose for DNA electrophoresis	white powder	1.7 g	2 vials	1.7 g	3 vials

EPh detection agarose kit variant 200 is intended for 240 samples (100 ml of gel is sufficient for 5 rows of 24 wells).

EPh detection agarose kit variant 300 is intended for 360 samples (100 ml of gel is sufficient for 5 rows of 24 wells).

EPh detection agarose kit variants genotype-200 or genotype-300 includes:

Reagent	***Description***	***Variant genotype-200***		***Variant genotype-300***	
		Volume (ml), Mass (g)	***Quantity***	***Volume (ml), Mass (g)***	***Quantity***
Tris-borate buffer (TBE) concentrated with ethidium bromide	orange clear liquid	50 ml	1 vial	75 ml	1 vial
High-resolution agarose for DNA electrophoresis	white powder	3.0 g	2 vials	3.0 g	3 vials

EPh genotype-200 and genotype-300 variants are intended for separation of PCR products in a “multiplex” format, when a single amplification product contains DNA fragments of different lengths.

EPh detection agarose kit variant genotype-200 is intended for 144 samples (100 ml of gel is sufficient for 3 rows of 24 wells).
EPh detection agarose kit variant genotype-300 is intended for 216 samples (100 ml of gel is sufficient for 3 rows of 24 wells).

4. ADDITIONAL REQIUREMENTS

- Horizontal electrophoresis chamber (up to 400-ml volume).
- Constant-current source with 150–460 V voltage.
- UV transilluminator with a room for gel scanning.
- Digital camera for data recording and image transferring.
- Water distiller.
- Refrigerator for 2–8 °C.
- Microwave oven.
- Conical 250-ml heat-proof flask.
- Graduated cylinder (1 L).
- Tube rack.
- Parafilm.
- Pipettes (adjustable).
- Pipette tips (up to 200 µl).
- Disposable powder-free gloves and laboratory coat.
- Waste bin for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all samples and unused reagents in compliance with local authorities requirements.

- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant, such as 0.5% sodium hypochlorite or another suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa. If skin, eyes, and mucosa contact, immediately flush with water and seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- The laboratory process must be one directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.

6. WORKING CONDITIONS

EPh detection agarose kit should be used at 18–25 °C.

7. PROTOCOL

7.1. Preparation of working buffer solution and agarose gel

7.1.1. Buffer for electrophoresis

Add **25 ml** of **Tris-borate buffer (TBE) concentrated with ethidium bromide** to a graduated cylinder. Then add **distilled water** to **500 ml**, close the cylinder with Parafilm, and mix.

Ethidium bromide is a carcinogenic substance. Use it in compliance with general precautions. All reagents contained ethidium bromide should be utilized in compliance with local authorities' requirements.

7.1.2. Agarose gel

1. Transfer agarose powder from the vial to a 250-ml heat-proof flask. Then add 100 ml of the prepared buffer, stir, and melt agarose in a microwave oven until it is completely dissolved. Agarose melts for 1.5 min at 800 W if 1 flask is placed in the oven (or 5 min if 5 flasks are placed).
2. Take out the flask with melted agarose from the microwave and mix it carefully by rotating the flask. Then place the flask into the microwave oven once again for 1.5 min (800W) and heat agarose to boiling. Take out the flask from the microwave oven and chill agarose to 65–70°C by rotating the flask.
3. Level the table for filling with gel. Fill up the melted gel into the form of horizontal electrophoresis chamber. Insert combs into the gel without touching the form bottom. The distance between combs should be not less than 3 cm or 5 cm from each other (variant

200/300 or variant genotype-200/genotype-300, respectively). Gel must be about 6 mm thick.

4. After the gel has completely polymerized (~ 30 minutes at room temperature), remove the combs carefully without damaging the wells. Transfer the support with the prepared gel into the horizontal electrophoresis chamber. Wells are to be placed nearer to the negative electrode (DNA will move to the positive electrode). Add the prepared buffer to the chamber ensuring that it covers the gel by 5 mm above.

7.2. Procedure

1. Tubes with amplification products are to be placed in the tube racks. Take **10-15 µl** of amplified DNA samples from under the oil and load into gel wells (if you use one tip for different samples, wash it with the buffer after each sample).

Each gel row should necessarily include Positive Control (C+) and advisably include a molecular-weight marker (not supplied with the EPh detection agarose kit).

2. Connect the power supply with the chamber keeping the polarity (DNA moves towards the positive electrode) and switch the power supply on. If an SE-2 (Helicon) chamber for electrophoresis and an Elf-4 (DNA-Technology) power supply are used, set the following parameters: voltage, 250 V; mode, voltage stabilization; electrophoresis time, 18–20 min and 40 min (for variants 200/300 and genotype-200/genotype-300, respectively). The optimum electric-field strength under these conditions is 10 V/cm.
3. When the dye xylene cyanol reaches approximately half of the gel length (1.5 cm and 2.5 cm for variants 200/300 and genotype-200/genotype-300, respectively) and the dye cresol red reaches approximately 2/3 of the gel length (2 cm and 3.5 cm for variants 200/300 and genotype-200/genotype-300, respectively), switch the current source off and transfer the gel to the transilluminator, placing strips horizontally with wells up. Obtain an image of the gel in the computer using a video system and add the image to the database (make sure that the sample order is correct).

Protect eyes and skin from ultraviolet with a safety mask or a glass cover when operating any transilluminators.

Boric acid crystals may occur in the solution of Tris-borate buffer (TBE) concentrated with ethidium bromide if stored. It has no effect on the quality of the reagent.
To eliminate the crystals, prepare the working buffer solution (as described above) and then incubate it in a boiling water bath for 30 min stirring every 10-15 min.

Avoid undissolved boric acid crystals in the agarose gel.

If you have any questions or if you encounter problems, please contact our Authorized representative in the European Community.

8. TRANSPORTATION

EPh detection agarose kit should be transported at 2–25 °C.

9. STABILITY AND STORAGE

All components of EPh detection agarose kit are to be stored at 18–25 °C when not in use. All components of EPh detection agarose kit are stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

Keep away from light.

10. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **EPh** detection agarose kit has been tested against predetermined specifications to ensure consistent product quality.

11. KEY TO SYMBOLS USED

	Catalogue number		Caution
	Batch code		Sufficient for
	*In vitro* diagnostic medical device		Expiration Date
	Version		Consult instructions for use
	Upper limit of temperature		Keep away from sunlight
	Manufacturer		Authorised representative in the European Community
	Date of manufacture		

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01.07.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”