



For *in Vitro* Diagnostic Use

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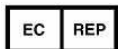
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EPh

Detection agarose kit

Instruction Manual

AmpliSens®



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1. INTENDED USE.

EPh detection agarose kit is 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 variant 200 or 300 includes:

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 for 5 lines x 24 holes).

EPh detection agarose kit variant 300 is intended for 360 samples (100 ml of gel is for 5 lines x 24 holes).

EPh detection agarose kit variant 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 “multiplex” format when a single amplified sample contains DNA fragments on different lengths.

EPh detection agarose kit variant genotype-200 is intended for 144 samples (100 ml of gel is for 3 lines x 24 holes).

EPh detection agarose kit variant genotype-300 is intended for 216 samples (100 ml of gel is for 3 lines x 24 holes).

4. ADDITIONAL REQUIREMENTS.

- Horizontal electrophoresis chamber of not more than 400 ml volume
- Constant-current source with 150-460 V voltage
- UV transilluminator with room for gel scanning
- Digital camera for results registration and image transmission
- Distiller for water
- Refrigerator with temperature between 2 and 8 °C.
- Microwave oven for agarose melting
- Conical heat-proof flask of 250 ml volume for agarose melting
- Graduated cylinder of 1 litre volume
- Tube rack
- Parafilm
- Pipettes (adjustable)
- Pipette tips (up to 200 µl)
- Plastic container for deactivation of buffers and gels that contains ethidium bromide.
- Disposable powder-free gloves and laboratory coat

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 other suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa contact immediately flush with water, seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one directional, it should begin in the Extraction Area move to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which the previous step was performed.



Some components of this kit contain Sodium Azide as a preservative. Do not use metal tubing for reagent transfer.



Xn

Tris-borate buffer (TBE) concentrated with ethidium bromide

Contains ethidium bromide: harmful.
Risk and safety phrases*
R22-26-36/37/38-68;
S26-28-36/37/39-45

*R22: Harmful if swallowed

R26: Very toxic by inhalation

R36/37/38: Irritating to the eyes, respiratory system and skin

R68: Possible risk of irreversible effects

S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice

S28: After contact with skin, wash immediately with plenty of water

S36/37/39: Wear suitable protective clothing, gloves and eye/face protection

S45: In case of accident or if you feel unwell, seek medical advice immediately (show label where possible)

6. PROTOCOL.

6.1. Preparation of working solutions and agarose gel.

6.1.1. Buffer for electrophoresis.

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

6.1.2. Agarose gel.

1. Transfer 1.7 g of agarose powder into the heat-proof flask of 250 ml volume. Then add 100 ml of prepared buffer, stir, and melt in microwave oven until agarose is completely dissolve. Agarose melting time is 1.5 min in 800W microwave oven if loaded with 1 flask (or 5 min - if 5 flasks are loaded).
2. Take out the flask with melted agarose from microwave and mix carefully by rotating. Then place the flask into the microwave oven for 1.5 min (800W) and boil agarose. Take out the flask from the microwave oven and cool to 65-70°C by rotating.
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 of 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 thickness must be about 6 mm.
4. After the gel has completely thickened (about 30 minutes at room temperature), remove the combs carefully without damaging of the holes. Transfer the padding with prepared gel into the

horizontal electrophoresis chamber. Holes are to be placed nearer to the negative electrode (DNA will move to the positive electrode). Add prepared buffer into the chamber ensuring it covers gel by 5 mm above.

6.2. Procedure.

1. Tubes with amplification's products are to be placed on the tube racks. Take **10-15 µl** of amplified DNA samples from under the oil and place into gel holes (if you use one tip for different samples then you need to wash it by buffer after each sampling).



Molecular weight marker (not supplied with Eph detection kit) and Positive Control (C+) should be present in each holes line.

2. Turn on power supply to the chamber keeping the polarity (DNA moves to the positive electrode) and switch the power supply on. If using "SE-2" (Helicon) chamber for electrophoresis and "Elf-4" (DNA-Technology) power supply apply following parameters: voltage - 250 V, voltage stabilization, electrophoresis time - 18-20 min or 40 min (variant 200/300 or variant genotype-200/genotype-300, respectively). Optimum electric-field strength is 10 V/cm in such conditions.
3. When dyes: xylencyanol reaches approximately half of the gel length - 1.5 cm or 2.5 cm (variant 200/300 or variant genotype-200/genotype-300, respectively); cresol red reaches approximately 2/3 of the gel length - 2 cm or 3.5 cm (variant 200/300 or variant genotype-200/genotype-300, respectively) switch current source off, and transfer the gel on the transilluminator, placing strips horizontally with holes up. Get the gel image on the computer by using of video system, register the samples order and add the image to database.



Put the protective mask or use the glass filter while watching and photographing the gel.



While storing Tris-borate buffer (TBE) concentrated with ethidium bromide it's possible to precipitate the boric acid salts. It has no affect on the quality of the reagent. If the precipitation occurs in concentrated buffer then the working solution (as described above) is prepared. Then it's heated at bain-marie for 30 min with stirring every 10-15 min until the precipitation is completely dissolved.



Avoid undissolved grains of boric acid in agarose gel.

7. STABILITY AND STORAGE.

All components of Eph detection agarose kit are to be stored at the temperature between 18 °C and 25 °C when not in use. All components of Eph detection agarose kit are to be stable until labeled expiration date.















Keep away from light.

8. QUALITY CONTROL.

In accordance with Federal State Institution of Science “Central Research Institute of Epidemiology” ISO 13485 – certified Total Quality Management System, each lot of EPh detection agarose kit is tested against predetermined specifications to ensure consistent product quality.

9. EXPLANATION OF SYMBOLS.

	Manufacturer		Temperature limitation
	Use by		Batch code
	For <i>in Vitro</i> Diagnostic Use		Version
	Catalogue number		Authorized representative in the European Community.
	Contains sufficient for <n> tests		Caution, consult accompanying documents
	Consult instructions for use		Harmful