HIGH QUALITY CIRCULATING DNA EXTRACTION KIT FROM SERUM OR PLASMA SAMPLES

General instructions:

To ensure proper use and handling, please, READ THE ENTIRE MANUAL BEFORE using the Kit.

Labelling the top of each vial upon arrival of the kit is highly recommended to avoid mistakes.

This DNA Kit has been designed for in vitro diagnostic use.

The approximate processing time after proteinase K digestion is 45 minutes.

Kit contents

	20 extractions kit	50 extractions kit
Proteinase K	0.65 ml	1.6 ml
Solution A	2.1 ml	5.2 ml
Solution B	4.5 ml	10.5 ml
Solution C	1.7 ml	4.2 ml
Solution D	12.5 ml	32 ml (2 x 16 ml)
Solution E	12 ml	25.5 ml
Solution F	1.5 ml	3 ml
Microtubes (1.5 ml)	40	100

Equipment and materials required but not supplied

The following equipment and materials are required:

Pipets and pipet tips (to prevent cross-contamination, pipet tips with aerosol barriers are strongly recommended)

Disposable gloves

Heating block for lysis of samples at 56ºC

Microcentrifuge

Vortexer

Technical considerations

This Kit was specially designed with the aim of obtaining, in a reproducible manner, circulating DNA from as little as 0.5 ml serum or plasma samples, for subsequent uses in genetic or epigenetic analysis procedures. Other cell free body fluids such as urine can also be used to obtain circulating DNA with this kit.

Smaller sample volumes can be used, reducing solutions volumes in the same ratio. For larger volumes, divide into 0.5 ml aliquots and proceed to DNA purification separately. Purified DNA from the same sample aliquots can be mixed at the end of the procedure, but this step increases final volume.

It is important to notice that circulating DNA is often present in very low concentrations (1-100 ng/ml in healthy people) and as short fragments, <1000 bp. The concentration also varies considerably among different individuals. However, purified DNA using this kit, has been successfully analyzed in different applications such as genotyping experiments and PCR amplifications.

Identification of the substance/mixture

SOLUTION A

Non Hazardous

SOLUTION B:

Non Hazardous

SOLUTION C: Contains sodium acetate solution

1. HAZARDS IDENTIFICATION

a. Classification of the substance or mixture

- **Classification according to Regulation (EC) No 1272/2008**

Eye irritation (Category 2)

Not a hazardous substance or mixture according to EC-directives 67/548/EEC or 1999/45/EC.

b. Label elements

2. HANDLING

a. Precautions for safe handling

Avoid contact with skin and eyes. Avoid inhalation of vapour or mist.

Normal measures for preventive fire protection.

3. ACCIDENTAL RELEASE MEASURES

a. Personal precautions, protective equipment and emergency procedures

Use personal protective equipment. Avoid breathing vapors, mist or gas.

Ensure adequate ventilation.

b. Environmental precautions

Do not let product enter drains.

c. Methods and materials for containment and cleaning up

Soak up with inert absorbent material and dispose of as hazardous waste. Keep in suitable, closed containers for disposal.

SOLUTION D: Contains 2-Propanol

1. HAZARDS IDENTIFICATION

a. Classification of the substance or mixture

- **Classification according to Regulation (EC) No 1272/2008 [EU-GHS/CLP]**

Flammable liquids (Category 2)

Eye irritation (Category 2)

Specific target organ toxicity - single exposure (Category 3)

- **Classification according to EU Directives 67/548/EEC or 1999/45/EC**

Highly flammable. Irritating to eyes. Vapours may cause drowsiness and dizziness.

b. Label elements

2. HANDLING

a. Precautions for safe handling

Avoid contact with skin and eyes. Avoid inhalation of vapour or mist.

Keep away from sources of ignition - No smoking.Take measures to prevent the build up of electrostatic charge.

b. Skin protection

Handle with gloves. Gloves must be inspected prior to use.

Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices.

Wash and dry hands.

3. ACCIDENTAL RELEASE MEASURES

a. Personal precautions, protective equipment and emergency procedures

Use personal protective equipment. Avoid breathing vapors, mist or gas.

Ensure adequate ventilation. Remove all sources of ignition.

Evacuate personnel to safe areas. Beware of vapours accumulating to form explosive concentrations.

Vapours can accumulate in low areas.

b. Environmental precautions

Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

SOLUTION E

Non Hazardous

SOLUTION F

Non Hazardous

Storage

Keep container tightly closed in a well-ventilated place.

All buffers can be stored at room temperature (15-25ºC). If temperature exceeds 25ºC, is recommended to storage at least solution A, B and C, in a cool place (2-8ºC).

Proteinase K is also stable at room temperature but storage at 2-8ºC is recommended to prolong its lifetime.

Remember, if stored at (2-8ºC) solutions should be homogenized and equilibrated to room temperature before use (especially solution A to dissolve white precipitated formed).

All buffers are stable for at least 1 year when stored at room temperature (15-25ºC) but only until the kit expiration date (see box label). If stored at 2-8ºC the kit is stable for more than 1 year and quality does not decrease.

Procedure recommendations

- Samples

It is important to process blood samples appropriately in order to minimize contamination by cellular DNA in plasma samples. **An additional high-speed spin in a bench microcentrifuge is recommended prior to storage and/or DNA extraction.** For urine samples this step is also recommended in order to avoid the presence of epithelial cells that could contaminate the sample.

The DNA extraction procedure can be performed from fresh or frozen serum/plasma or urine samples.

- Solutions

Gently homogenize every solution before use. White precipitates can appear in solution A when store at 2-8ºC or even at room temperature. Homogenization at 55ºC is recommended before use.

DNA Quantity

Concentration

Circulating DNA in cell free body fluids is present in very low concentrations and are therefore difficult to determine with spectrophotometers. DNA quantity in the purified sample must be determined by fluorometric based method in order to get accurate and reliable concentration readings (e.g. PicoGreen® based methods).