

[Introduction]

SMITEST EX-R&D is a kit to extract nucleic acids from viruses rapidly and easily from serum mainly. It is also able to use for the extraction of nucleic acids from nucleated cells of peripheral blood, cultured cells or others. The purified nucleic acids are useful for the material of various genetic engineering studies such as PCR (Polymerase Chain Reaction), reverse transcription etc.

Current extraction or purification methods for nucleic acids have been required complex and dangerous operation, because hazardous chemical solvent of phenol and/or chloroform were necessary to extract. In addition, contamination or mistake of order of samples was problem, as transfer works of the aqueous solution from tube to tube have been frequently needed.

SMITEST EX-R&D is free from phenol or chloroform, and realized "single tube extraction" to operate following three step simple operation. "Single tube extraction" makes not only improvement for procedure simplified, but also high yield and multiple specimen extraction.

[Principle]

First step: (Enzyme treatment for serum specimens)

Non specific proteins in serum are treated to degrade by enzyme, detergent and reduction agent.

Second step: (Disposition by protein dissolving agent)

Proteins in serum are denatured and solubilized by chaotropic agent.

Third step: (Precipitation of nucleic acids by alcohol)

Nucleic acids existing in solution are precipitated by 2-propanol and GSL's original Precipitator.

[Composition of the kit]

Code No.	Name	Contents	Volume	Quantity
EX-01	Solution I	Enzyme Solution	1550 ul	1 vial
EX-02	Solution II	Specimen Diluent	49 ml	1 vial
EX-03	Solution III	Protein Dissolvent	41 ml	1 vial
EX-04	Solution IV	Precipitator	550 ul	1 vial

[Intended use]

EX-R&D is a reagent for the extraction of nucleic acids from viruses etc. existing in serum, plasma (treated with EDTA or citrate) and from nucleated cells in peripheral blood or cultured cells.

[Procedure]

1. Extraction of nucleic acids from serum or plasma specimen
 - 1-1. Necessary reagents, instruments not supplied in the kit
 - . 2-propanol (isopropanol), super (special) grade
 - . 70 % of Ethanol

- . 1.5 ml or 2.0 ml of micro tube for centrifugation
- . Centrifugation apparatus (12,000 x g)
- . Incubator (Block heater or Air incubator at 55 degrees centigrade)
- . Vortex mixer
- . Micropipette
- . Tips with filter
- . Desiccator, Water-jet pump etc.

1-2. Operation procedure (for 1.5 ml of centrifugation micro tube)

- (1) Dispense 15 micro l of Enzyme Solution (Solution I), 380 micro l of Specimen Diluent (Solution II) and 5 μ l of Precipitator (Solution IV) to cleaned 1.5ml of micro centrifugation tube. #1)
- (2) Add 10 to 100 micro l of serum specimen into the tube dispensed as above. #2)
- (3) Mix the tube with Vortex mixer and incubate for 30 minutes at 55 degrees centigrade. #3)
- (4) Add 250 micro l of Protein Dissolvent (Solution III) into the tube and mix with Vortex, then incubate for 15 min. at 55 degrees centigrade.
- (5) Add 600 micro l of 2-propanol into the tube and mix upside own thoroughly, then place over 15 min. at 4 degrees centigrade or on ice. #4)
- (6) Centrifuge at 12,000 x g for 10 min. at 4 degrees centigrade. Remove the supernatant by aspirator or pipette. #5)
- (7) Add 500 micro l of 70 % ethanol into the tube and after gently mixing, then, centrifuge the tube at 12,000 x g for 3 min. at 4 degrees centigrade. Remove the supernatant similarly as above. Repeat this washing step again (twice totally). #6)
- (8) Dry up obtained white precipitate pellet under vacuum (for 5 - 10 min.) or air ventilation (for approx.15 min.). #7)
- (9) Dissolve the precipitate pellet again with buffer solution or distilled water (free from DNase/RNase) suitable for experiment purpose.

- #1) It s possible that an aliquot of each three solution will be taken and mixed, then transferred to the tube.
- #2) Be careful for dispersion of the sample when the tube cap or specimen container is opened or shut. To be used filtered tip for pipetting.
- #3) In steps after (3) described above, short centrifugation is recommended before and/or after incubation to avoid dispersion of samples at opening the tube cap.
- #4) It is not necessary to return to room temperature 2-propanol at the time of adding.
- #5) Since the pellet is still translucent at that time, be careful to suck the semitransparent pellet when the supernatant is eliminated by aspiration.
- #6) After adding 70% ethanol, the precipitate becomes distinct white solid-like pellet. Perform decantation of the supernatant after second time washing as perfectly as possible.
- #7) Perform at site under constant air flow such as clean bench in case of air drying.

1-3. Operation procedure (for 2.0 ml of micro centrifugation tube)

Extraction for purification will be performed similarly as described above for ordinal serum specimen. However, in case of specimen considered to contain rich protein, the following method is recommended to extract for purification using 2.0 ml micro centrifugation tube.

No alteration points for incubation temperature and time, centrifugation condition, and

adding volume of 70% ethanol.

Enzyme Solution (Sol. I)	: 15 ul	Protein Dissolvent (Sol. III):	400 ul
Specimen Diluent (Sol. II)	: 480 ul	2-propanol	: 800 ul
Precipitator (Sol. IV)	: 5 ul		

(This kit includes enough volume of reagents to perform the extraction operation, if used for this 2.0 ml tube method.)

2. Extraction for nucleic acids from nucleated cells in peripheral blood or cultured cells
(Extraction of genomic DNA)

2-1. Necessary reagents, instruments not supplied in the kit

(# In addition to the above 1-1, the followings will be needed)

- . Centrifugation apparatus (2,000 x g)
- . Red cell lysis buffer (RCLB), Composition: 0.32M Sucrose, 1 v/v% Triton X-100, 5mM MgCl₂, 12 mM Tris-HCl (pH 7.5) (It is convenient to prepare this reagent which is condensed 5 times, and store at 4 degrees centigrade.)

(# RCLB is sold separately. SMITEST 5 x conc. RCLB solution: Product Code No. Q-02)

2-2. Operation procedure

- (1) Centrifuge 1 - 3 ml of peripheral blood which collected by EDTA blood collection tube under 2,000 x g, for 10 min. at 4 degrees centigrade. #1)
- (2) Discard the plasma portion being intact red blood cell layer.
- (3) Transfer buffy coat (upper layer part of blood cells layer, approximately 0.5 - 1.0 ml) to the centrifugation micro tube (2.0 ml of volume) previously added 1.0 ml of RCLB.
- (4) Mix well the tube with Vortex mixer.
- (5) Centrifuge the tube at 12,000 x g for 10 min. at 4 degrees centigrade, then discard the supernatant.
- (6) Add 1.0 ml of RCLB into the tube again and mix well, then centrifuge similarly as above and discard the supernatant. #2)
- (7) Add 480 micro l of Specimen Diluent (Sol. II) and 15 micro l of Enzyme Solution (Sol. I) into the tube. #3)
- (8) Mix the tube with Vortex mixer, then incubate for 30 min. at 55 degrees centigrade. #4), #5)
- (9) Add 400 micro l of Protein Dissolvent (Sol. III) into the tube and mix, then for 15 min. at 55 degrees centigrade.
- (10) Add 800 micro l of 2-propanol into the tube, and then mix upside down the tube thoroughly. #6)
- (11) Centrifuge tube at 12,000 x g, for 10 min. at 4 degrees centigrade. Remove the supernatant using aspirator or pipette.
- (12) Add 500 micro l of 70% ethanol into the tube and mix gently. Centrifuge at 12,000 x g for 3 min. at 4 degrees centigrade. Remove the supernatant similarly as above. Repeat this washing step again (twice totally).
- (13) Dry up the precipitate pellet obtained under vacuum (5 - 10 min.) or under ventilation (approx. 15 min.). #7)
- (14) Dissolve the pellet again with buffer or distilled water (free from DNase/RNase) suitable for purpose of the experiment. #8)

- #1) Collection method for nucleated cells is "method for collecting buffy coat" and "method using Ficoll", and either method is available. Here, "method for collecting buffy coat" is described as a simple method.
 - #2) White precipitate (pellet) of the nucleated cells will be obtained.
 - #3) Use the Precipitator in case recovered nucleic acids would be considered to be very little (below several micro grams).
 - #4) Should not mix vigorously in all operations after (8), if the high-molecular-weight DNA would be needed.
 - #5) Mix the tube sometimes during incubation at 55 degrees centigrade to accelerate dissolution of the precipitate.
 - #6) String-like DNA is precipitated by 2-propanol in case of large amount of extracted DNA.
 - #7) Excess vacuum drying would make it difficult to re-dissolve of the extracted DNA.
 - #8) In some cases the high-molecular-weight DNA is hard to re-dissolve. In such a case, proper incubation at 55 degrees centigrade is needed to accelerate dissolving.
- Extraction from cultured cells is performed as follows.

- (1) Wash the cultured cells (10^4 - 10^6 cells) with saline.
 - (2) Add 480 micro l of Specimen Diluent (Sol. II) and 15 micro l of Enzyme Solution (Sol. I) into the tube, then conduct the same operations after 2-2 (8) described above. #1)
- #1) Depending on type or amount of the cell, preliminary experiment would be required for setting the conditions such as required volume for each reagent.

[General precautions]

- (1) U-shaped bottomed micro tube is more preferable than V-shaped one, since in case of using the latter one the precipitate is not easily visible, and furthermore it tends to disperse or peel off.
- (2) Extraction sometimes does not go well in specimens containing high concentration of protein, very high or very low concentration of salts, or repeated freeze-thaw cycle many times.
- (3) Do not interrupt extraction operation on the way.
- (4) Store this kit at 2 - 8 degrees centigrade. Particularly, store Enzyme Solution (Sol. I) securely at 2 - 8 degrees centigrade to avoid inactivation, and do not place it for long time at room temperature.
- (5) Specimen Diluent (Sol. II) stored for long time at 2 - 8 degrees centigrade often causes white viscous liquid, however, it is not abnormal. Returning to room temperature, it will soon become transparent liquid. Use it in transparent liquid state.
- (6) Protein Dissolvent stored for long time below 4 degrees centigrade causes crystal sometime, however, it is not abnormal. In such a case, it will become transparent liquid after heating at around 55 degrees centigrade and mixing well. Use it after crystal will be perfectly dissolved.

[Precautions on handling]

- (1) This kit does not contain hazardous chemicals. But, it contains reagents to disintegrate and/or degenerate protein. Take fully care of yourself not to touch it to skin, mucous membrane, eyes and clothes. Wear gloves, eye protector at time of handling. If some reagent adheres on, wash out it by large volume of water, and consult by doctor if needed.

- (2) To protect contamination of DNase/RNase, be careful for wearing gloves during the operations and closing tightly cap of reagent bottles. Furthermore, to protect contamination by PCR amplified products, differentiate instruments (such as pipettes, centrifugation apparatus) for treatment for PCR amplified products from instruments for extraction operation absolutely.
- (3) To protect cross-contamination between specimens, pipette tips for extraction operation should always be prepared ones with filter.
- (4) This kit must be used within expired date. Do not mix reagents between different lots.

[Storage] 2 - 8 degrees centigrade

[Expired Date] 12 months after manufactured

[Package] 100 tests

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