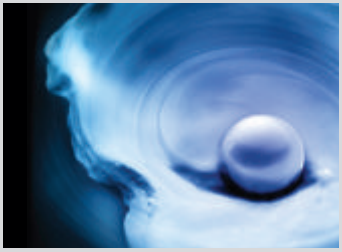

ISOLATE II
Genomic DNA Kit

Product Manual

BIOLINE

A Meridian Life Science® Company

ISOLATE II Genomic DNA Kit

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General Information

1. KIT CONTENTS

COMPONENT	10 Preps	50 Preps	250 Preps
ISOLATE II Genomic DNA Spin Columns (green)	10	50	250
Collection Tubes (2ml)	20	100	500
Lysis Buffer GL	5ml	20ml	100ml
Buffer G1	6ml	12ml	60ml
Buffer G2	1.5ml	3ml	15ml
Wash Buffer GW1	6ml	30ml	2 x 75ml
Wash Buffer GW2† (concentrate)	4ml	2 x 7ml	2 x 40ml
Elution Buffer G	3ml	15ml	75ml
Proteinase K (lyophilized)	6mg	30mg	2 x 75mg
Proteinase K Buffer PR	0.8ml	1.8ml	8ml
Labels for Lysis Buffer G3	1	1	1
User Manual	1	1	1
Bench Protocol Sheet	1	1	1

† Before use, add indicated volume of 96-100% ethanol and mark wash buffer bottle label.

2. DESCRIPTION

The ISOLATE II Genomic DNA Kit is a simple, reliable and fast method for isolation of high-quality genomic DNA from a variety of sample sources.

Biological samples are first lysed in chaotropic salt ions in the presence of Proteinase K. Ethanol is added to the sample and then processed through a genomic DNA mini spin column containing a silica membrane to which the genomic DNA binds. Contaminants and impurities such as salts, metabolites and cellular components are effectively removed by simple washing steps with two different buffers. High-quality purified genomic DNA is then eluted in an elution buffer.

Please read this manual carefully to familiarize yourself with the ISOLATE II Genomic DNA protocol before starting (also available on www.bioline.com). More experienced users can refer to the bench-top protocol for quick referencing during the procedure.

3. STORAGE

Dissolved Proteinase K solution is stable at-20°C for up to 6 months. All other kit components should be stored at room temperature (18–25°C) and are stable for up to 1 year. Storage at lower temperatures may cause precipitation of salts in Buffers GL, G1 or G3. Incubate bottle at 50-70°C prior to use to dissolve precipitates.

4. SAFETY INFORMATION

When working with chemicals, always wear a suitable lab coat, gloves and safety glasses.

Buffer G1 and Wash Buffer GW1 contain guanidine hydrochloride. This chemical is harmful when in skin contact, inhaled or ingested.

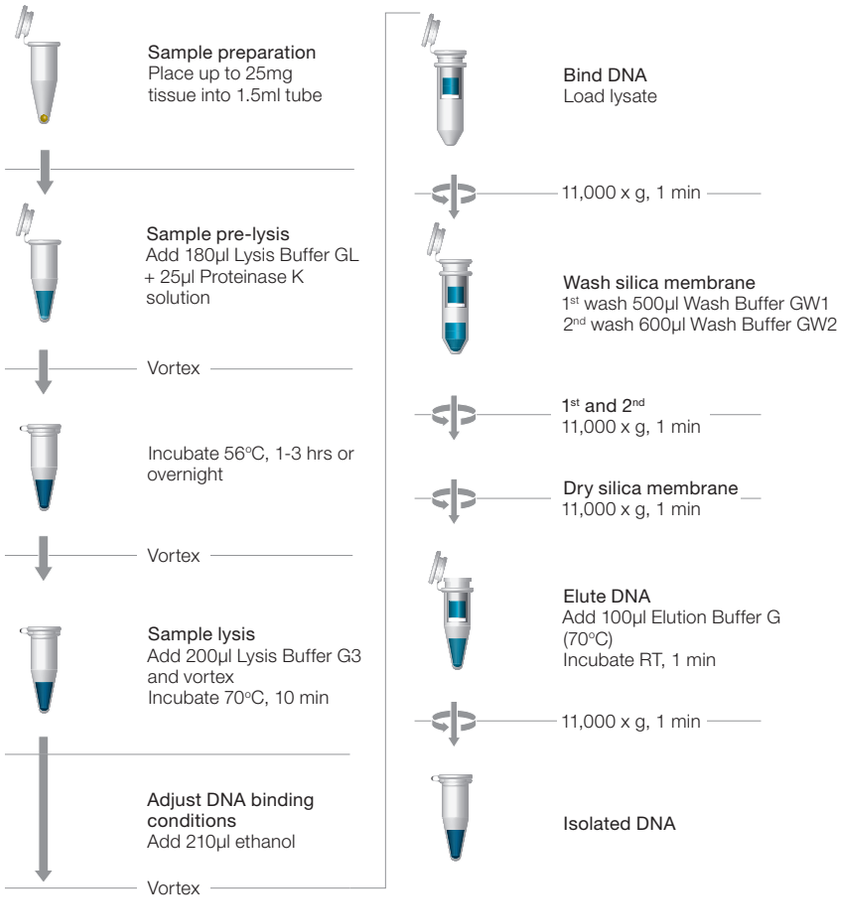
For detailed information, please consult the material data safety sheets (MSDSs) available on our website at www.bioline.com.

5. PRODUCT SPECIFICATIONS

The ISOLATE II Genomic DNA Kit is specially designed for the rapid and efficient isolation of extremely pure genomic DNA from any tissue, cells, bacteria, yeast, forensic samples, serum, plasma, or other body fluids. The hands on time is 20 min for 4-6 preps following the lysis steps. The isolated DNA is of high purity (A_{260}/A_{280} ratio: 1.7-1.9) with yields of 20-35µg (see below).

ISOLATE II GENOMIC DNA COLUMN SPECIFICATIONS	
Max. binding capacity	60µg DNA
A_{260}/A_{280}	1.7-1.9
Typical yield	20–35µg
Elution volume	60-100µl
Sample material	
Tissue	1–25mg
Cells	10^2–10^7

Genomic DNA Isolation



6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

When working with chemicals, always wear a suitable lab coat, protective goggles and disposable gloves.

- 96-100% ethanol† (for Wash Buffer GW2)
- Microcentrifuge tubes (1.5ml)
- Sterile DNase-free tips
- Pipettes
- Microcentrifuge (capable of 11,000 x g)
- Vortex mixer
- Thermal heating block
- Equipment for sample disruption and homogenization
- Personal protection equipment (lab coat, gloves, goggles)

† Molecular biology grade ethanol is recommended. Do not use denatured alcohol which contains unwanted additives such as methanol and acetone.

7. IMPORTANT NOTES

7.1 BUFFER PREPARATION AND PARAMETERS

Preparing Lysis Buffer G3

Transfer the contents of Buffer G1 to Buffer G2. Mix well and label Lysis Buffer G3 on the bottle.

Note: The resulting Lysis Buffer G3 is stable for up to one year at room temperature. If a white precipitate forms in Lysis Buffer G3 at any time, re-dissolve by incubating the bottle at 70°C before use.

Preparing Wash Buffer GW2

Add 96–100% ethanol to Wash Buffer GW2 Concentrate: 16ml for the 10 prep kit, 28ml for the 50 prep kit and 160ml x 2 for the 250 prep kit.

Note: Mark bottle label to indicate ethanol was added. Store Wash Buffer GW2 at room temperature for up to 1 year.

Preparing Proteinase K Buffer PR

Add Proteinase K Buffer PR to the lyophilized Proteinase K: 260µl for the 10 prep kit, 1.35ml for the 50 prep kit and 3.35ml x 2 for the 250 prep kit.

Note: Proteinase K solution is stable at-20°C for up to 6 months.

Elution parameters

It is possible to modify the elution protocol to improve yield and concentration.

Use Elution Buffer G preheated to 70°C for one of the following procedures:

- High yield: Two elution steps with 100µl Elution Buffer G (to increase yield to 90–100%).
- High concentration: One elution step with 60µl Elution Buffer G (to increase concentration by about 130%). Maximal yield 80%.

- High yield and high concentration: Two elution steps. Add 50µl Elution Buffer G, incubate for 3 min and centrifuge, repeat with a second 50µl Elution Buffer G. Yield 85–100% at a high concentration.

DNA Storage

Store isolated DNA at-20°C. Several freeze-thaw cycles will not interfere with most downstream applications, however for long-range PCR or high sensitivity (especially in real-time PCR), store in aliquots to avoid multiple freeze-thawing.

8. STANDARD PROTOCOL

8.1 PURIFYING DNA FROM CULTURED CELLS AND HUMAN OR ANIMAL TISSUE

Before you start:

- Make sure Lysis Buffer G3, Wash Buffer GW2 and Proteinase K are prepared (see section 7.1).
- Set an incubator or water bath to 56°C.
- Preheat Elution Buffer G to 70°C.

1 **Sample preparation**

1.1 **Human or animal tissue**

Cut 25mg of tissue into small pieces. Place the sample in a 1.5ml microcentrifuge tube (proceed to step 2).

Note: Samples that are difficult to lyse can be ground under liquid nitrogen or may be treated in a mechanical homogenizer: Add 25mg of tissue to a 1.5ml microcentrifuge tube (not supplied), add 50–75µl PBS and homogenize.

1.2 **Cultured cells**

Resuspend up to 10^7 cells in a final volume of 200µl Lysis Buffer GL. Add 25µl Proteinase K solution and 200µl Lysis Buffer G3. Incubate the sample at 70°C for 10-15 min (proceed to step 4).

2 **Pre-lysis**

Add 180µl Lysis Buffer GL and 25µl Proteinase K solution, completely cover sample with solution and vortex.

Note: If processing several samples, Proteinase K and Lysis Buffer GL may be premixed directly before use (no more than 10–15 min before addition to the sample, as Proteinase K will self-digest in Lysis Buffer GL without substrate).

Incubate at 56°C for 1–3 hours (until completely lysed), shake or vortex occasionally.

Note: Samples can be incubated overnight. If RNA-free DNA is needed for downstream applications, an RNase digest may be performed (RNase not included).

3 **Lyse sample**

Vortex sample briefly and add 200µl Lysis Buffer G3. Vortex vigorously and incubate at 70°C for 10 min.

Note: If insoluble particles are visible, centrifuge for 5 min at high speed and transfer the supernatant to a new microcentrifuge tube.

4 **Adjust DNA binding conditions**

Vortex briefly and add 210µl ethanol (96-100%) to the sample. Vortex vigorously.

Note: After addition of ethanol a stringy precipitate may become visible. This will not affect the DNA isolation.

5 **Bind DNA**

For each sample, place an ISOLATE II Genomic DNA Spin Column into a Collection Tube. Add all of the sample to the column and centrifuge for 1 min at 11,000 x g. Discard the flow-through and reuse Collection Tube. Repeat at a higher g force if samples are not completely filtered through matrix.

6 **Wash silica membrane**

- Add 500µl Wash Buffer GW1. Centrifuge for 1 min at 11,000 x g. Discard flow-through and reuse Collection Tube.
- Add 600µl Wash Buffer GW2 to the column and centrifuge for 1 min at 11,000 x g. Discard flow-through and reuse Collection Tube.

7 **Dry silica membrane**

Centrifuge 1 min at 11,000 x g, to remove residual ethanol. Place the ISOLATE II Genomic DNA Spin Column in a 1.5ml microcentrifuge tube (not supplied).

8 **Elute DNA**

Add 100µl preheated Elution Buffer G (70°C) directly onto the silica membrane. Incubate at room temperature for 1 min. Centrifuge 1 min at 11,000 x g.

Note: For alternative elution procedures see section 7.1.

9. ALTERNATIVE PROTOCOLS

9.1 MOUSE OR RAT TAILS

Before you start:

- Make sure Lysis Buffer G3, Wash Buffer GW2 and Proteinase K are prepared (see section 7.1).
- Set an incubator or water bath to 56°C.
- Preheat Elution Buffer G to 70°C.

1 **Sample preparation**

Cut two 0.6cm pieces of mouse tail and place in a 1.5ml centrifuge tube (not supplied).

Note: For rat tails, one 0.6cm piece is sufficient.

2 **Pre-lysis**

Add 180µl Lysis Buffer GL and 25µl Proteinase K solution. Completely cover sample with solution and vortex.

Note: If processing several samples, Proteinase K and Lysis Buffer GL may be premixed directly before use (no more than 10–15 min before addition to the sample, as Proteinase K will self-digest in Lysis Buffer GL without substrate).

Incubate at 56°C overnight (or until completely lysed), shaking or vortexing occasionally.

To remove residual bone, hair etc., centrifuge for 5 min at 11,000 x g. Transfer 200µl supernatant to a new centrifuge tube.

3 **Lyse sample**

Vortex sample briefly and add 200µl Lysis Buffer G3. Vortex vigorously.

4 **Adjust DNA binding conditions**

Add 210µl ethanol (96–100%) to the sample. Vortex vigorously.

Proceed with step 5 of the standard protocol (see section 8.1).

9.2 BACTERIA

Before you start:

- Make sure Lysis Buffer G3, Wash Buffer GW2 and Proteinase K are prepared (see section 7.1).
- For hard-to-lyse bacteria make up the following lysis buffer (20mM Tris/HCl; 2mM EDTA; 1% Triton X-100; pH 8, supplemented with 20mg/ml lysozyme or 0.2mg/ml lysostaphin) (not supplied).
- Set an incubator or water bath to 56°C.
- Preheat Elution Buffer G to 70°C.

1 **Sample preparation**

Up to 1ml of bacterial culture can be used for the preparation depending on density, culture medium, bacterial strain etc.

Centrifuge up to 1ml culture for 5 min at 8,000 x g. Remove supernatant.

2 **Pre-lysis**

Resuspend pellet in 180µl Lysis Buffer GL and 25µl Proteinase K solution and vortex vigorously.

Incubate at 56°C for 1–3 hours (until completely lysed), shake or vortex occasionally.

Note: Samples can be incubated overnight. If RNA-free DNA is needed for downstream applications, an RNase digest may be performed (RNase not included).

Note: For hard-to-lyse bacteria such as Gram-positive bacteria, a preincubation is necessary: Resuspend the pelleted cells in a lysis buffer (instead of Lysis Buffer GL, see above) supplemented with lysozyme or lysostaphin and incubate for 30-60 min at 37°C. Add 25µl Proteinase K, incubate at 56°C until complete lysis is obtained.

Proceed with step 3 of the standard protocol (see section 8.1).

9.3 YEAST

Before you start:

- Make sure Lysis Buffer G3, Wash Buffer GW2 and Proteinase K are prepared (see section 7.1).
- Make up sorbitol buffer (1.2M sorbitol; 10mM $CaCl_2$; 0.1M Tris/HCl pH 7.5; 35mM ß-mercaptoethanol) (not supplied).
- Check that lyticase or zymolyase (not supplied) is available.
- Set an incubator or water bath to 30°C and 56°C.
- Preheat Elution Buffer G to 70°C.

1 **Sample preparation**

Centrifuge 3ml YPD yeast culture (OD_{600} ≤10) for 10 min at 5,000 x g. Remove supernatant.

Wash once with 1ml 10mM EDTA, pH 8 and centrifuge for 10 min at 5,000 x g.

2 **Pre-lysis**

Resuspend the pellet in 600µl sorbitol buffer and add 50U lyticase or zymolase. Incubate at 30°C for 30 min. This step degrades the yeast cell wall creating spheroplasts. Spheroplast formation may be checked microscopically.

Note: Concentration of lyticase or zymolyase can be increased up to 200U if spheroplasts are not found.

Centrifuge for 10 min at 2,000 x g and resuspend the pelleted spheroplasts in 180µl Lysis Buffer GL and 25µl Proteinase K solution and vortex vigorously. Incubate at 56°C for 1–3 hours (until completely lysed), shaking or vortexing occasionally.

Note: Samples can be incubated overnight. If RNA-free DNA is needed for downstream applications, an RNase digest may be performed (RNase not included).

Proceed with step 3 of the standard protocol (see section 8.1).

9.4 DRIED BLOOD SPOTS

Before you start:

- Make sure Lysis Buffer G3, Wash Buffer GW2 and Proteinase K are prepared (see section 7.1).
- Set an incubator or water bath to 56°C and 94°C.
- Preheat Elution Buffer G to 70°C.

1 **Sample preparation**

Cut out one or two dried blood spots (15 and 30mm² in area) as accurately as possible. Cut spots into small pieces and place into a 1.5ml microcentrifuge tube (not supplied).

2 **Pre-lysis**
Add 180µl Lysis Buffer GL and incubate at 94°C for 10 min.
Cool and add 25µl Proteinase K solution. Completely cover sample and incubate at 56°C for 60 min, shaking or vortexing occasionally.

3 **Lyse sample**
Vortex sample briefly and add 200µl Lysis Buffer G3. Vortex vigorously.
Proceed with step 4 of the standard protocol (see section 8.1).

9.5 GENOMIC/VIRAL DNA FROM BLOOD

Before you start:

- Make sure Lysis Buffer G3, Wash Buffer GW2 and Proteinase K are prepared (see section 7.1).
- Preheat Elution Buffer G to 70°C.

1 **Lyse blood**
Add 25µl Proteinase K, Buffer PR and 200µl sample into a 1.5ml microcentrifuge tube (not supplied).
Note: Make up sample to 200µl with PBS if using less volume. For cultured cells, resuspend up to 5 x 10^6 cells in 200µl PBS.
Add 200µl Lysis Buffer G3 and vortex vigorously for 10–20s.
Incubate samples at 70°C for 10–15 min.
Note: The lysate should turn brownish during incubation with Lysis Buffer G3. If processing older or clotted blood, increase Proteinase K incubation time up to 30 min and vortex vigorously several times during incubation.

2 **Adjust DNA binding conditions**
Add 210µl ethanol (96–100%) and vortex.

3 **Bind DNA**
For each preparation, place one ISOLATE II Genomic DNA Spin Column in a Collection Tube and load the sample onto the column. Ensure all lysate is loaded. Centrifuge for 1 min at 11,000 x g. Repeat at a higher g force if samples are not completely filtered through matrix. Place column in a new Collection Tube (2ml).
Proceed with step 6 of the standard protocol (see section 8.1).

9.6 HAIR ROOTS

Before you start:

- Make sure Lysis Buffer G3, Wash Buffer GW2 and Proteinase K are prepared (see section 7.1).
- Set an incubator or water bath to 56°C.
- Preheat Elution Buffer G to 70°C.

1 **Sample preparation**
Cut up to 100 hair roots from the hair sample and place in a 1.5ml centrifuge tube (not supplied).

2 **Pre-lysis**
Add 180µl Lysis Buffer GL and freeze the samples in liquid nitrogen. Thaw samples in a 56°C water bath. Repeat this freeze/thawing procedure 4 times.
Add 25µl Proteinase K solution and incubate at 56°C overnight (or until completely lysed), shake or vortex occasionally.
Proceed with step 3 of the standard protocol (see section 8.1).

9.7 PARAFFIN-EMBEDDED TISSUE

Before you start:

- Make sure Lysis Buffer G3, Wash Buffer GW2 and Proteinase K are prepared (see section 7.1).
- Check that n-octane or xylene (not supplied) is available.
- Set an incubator or water bath to 37°C and 56°C.
- Preheat Elution Buffer G to 70°C.

1 **Sample preparation**
Trim excess paraffin off the block and cut small sections (up to 25mg). With tweezers or toothpicks place the sections into microcentrifuge tubes (not supplied).
Add 1ml n-octane or xylene to each tube and vortex vigorously. Incubate at room temperature for 30 min, vortexing occasionally.
Centrifuge at 11,000 x g for 3 min. Discard supernatant.
Add 1ml ethanol (96–100%) and mix by inverting several times. Centrifuge at 11,000 x g for 3 min. Discard supernatant.
Repeat the ethanol washing step. Remove as much of the ethanol as possible.
Incubate the open tube at 37°C until the ethanol has evaporated (~15 min).
Proceed with step 2 of the standard protocol (see section 8.1).

9.8 GENOMIC DNA FROM FECAL MATERIAL

This protocol is suited for the isolation of genomic DNA from fecal material. Whilst this protocol is optimized for human cells and microorganisms, a supplementary protocol for viral DNA is also provided (see section 9.9).

Before you start:

- Make sure Lysis Buffer G3, Wash Buffer GW2 and Proteinase K are prepared (see section 7.1).

- Set an incubator or water bath to 37°C and 56°C.
- Preheat Elution Buffer G to 70°C.

1 **Sample preparation**

Add 250mg fecal material to 1ml TE buffer. Vortex vigorously (30s) to resuspend the sample.

Centrifuge for 15 min at 4,000 x g. Remove the supernatant.

Resuspend the pellet in 0.2-1ml Lysis Buffer GL. Add sufficient buffer to thoroughly resuspend the sample.

Transfer 200µl of the resuspended sample to a new microcentrifuge tube and add 25µl of Proteinase K. Incubate for 1-3 hours at 56°C.

Note: Cells from human, bacterial and pathogenic origin are found in fecal material and will lyse during the Proteinase K/Lysis Buffer GL incubation at 56°C with different efficiencies. To detect cells that are difficult to lyse (e.g. some bacteria and parasites) performing an additional incubation at increased incubation temperature (up to 95°C, 5-10 min) may help to increase DNA yield.*

Proceed to step 3 (lyse sample) of the standard protocol (see section 8.1).

**Release of bacterial/pathogen DNA can be monitored by using qPCR (or a similar technique) to examine the human/non-human ratio.*

9.9 VIRAL DNA FROM FECAL MATERIAL

This protocol is suited for the isolation of viral genomic DNA from fecal material.

Before you start:

- Make sure Lysis Buffer G3, Wash Buffer GW2, and Proteinase K are prepared (see section 7.1)
- Set an incubator or water bath to 56°C.
- Preheat Elution Buffer G to 70°C.
- Prepare 0.9% (w/v) NaCl in molecular biology grade water.

1 **Sample preparation**

Suspend the fecal sample (approx. 0.5g) in 0.9% NaCl solution (max.4ml).

Centrifuge the fecal sample (5 min at 800 x g) at room temperature. Filter the supernatant using a 0.22-0.45µm sterile filter. Centrifuge for 1 min at 11,000 x g.

2 **Pre-lysis**

Carefully decant the supernatant. Add 400µl Lysis Buffer GL, then 35µl Proteinase K and mix by vortexing. Transfer the supernatant into a sterile 1.5ml microcentrifuge tube (not supplied).

3 **Lyse sample**

Add 400µl Lysis Buffer G3 to the supernatant from step 2 and mix by vortexing. Incubate for a minimum of 30 min at 70°C.

4 **Adjust DNA binding conditions**

Add 420µl ethanol (96-100%) to the lysed sample from step 3 and mix by vortexing.

5 **Bind DNA**

For each sample, place one ISOLATE II Genomic DNA Spin Column into a Collection Tube and load the lysate. Centrifuge for 1 min at 4,500 x g. Discard the flow-through and return column into Collection Tube. Repeat lysate loading to the column and centrifugation steps if necessary.

If sample does not completely transfer through the silica membrane, repeat centrifugation step at 11,000 x g. Discard flow-through.

6 **Wash silica membrane**

- Add 600µl Wash Buffer GW1 to the column. Centrifuge for 1 min at 4,500 x g.Discard the flow-through and reuse Collection Tube.
- Add 600µl Wash Buffer GW2 to the column. Centrifuge for 1 min at 4,500 x g. Discard the flow-through and reuse Collection Tube.
- Add 600µl Wash Buffer GW2 to the column. Centrifuge for 2 min at 11,000 x g.

Discard the flow-through. Place ISOLATE II Genomic DNA Spin Column into a new Collection Tube.

7 **Dry silica membrane**

Incubate ISOLATE II Genomic DNA Spin Column with the lid opened for 1-2 min at 70°C (to remove residual ethanol). Place the column in a new 1.5ml microcentrifuge tube (not supplied).

8 **Elute DNA**

Add 100µl pre-warmed Elution Buffer G (70°C) to the column. Incubate for 3-5 min at 70°C with the lid closed. Centrifuge for 1 min at 4,500 x g.

For alternative elution procedures see section 7.1.

9 **PCR**

Use 10µl purified DNA as template in a 20µl PCR reaction.

Add an inhibition control mix (10µl purified DNA template with human DNA). Amplify with primers specific for a human DNA sequence such as ß-actin, ß-globin, or another reference gene of choice.

9.10 BACTERIAL DNA FROM URINE

This protocol is designed for purification of bacterial genomic DNA from urine samples.

Before you start:

- Make sure Lysis Buffer G3, Wash Buffer GW2, and Proteinase K are prepared (see section 7.1)
- Set an incubator or water bath to 56°C.
- Preheat Elution Buffer G to 70°C.

1 **Sample preparation**

Centrifuge a 1ml urine sample at 13,000 x g for 30 min. Discard the supernatant.

Add another 1ml of the urine sample to the pelleted material. Centrifuge at 13,000 x g for 30 min and discard supernatant.

Repeat the centrifugation with a third 1ml sample of urine and discard the supernatant.

Note: Fresh urine samples should be used. Storage at-20°C to-80°C is only recommended for 1-2 days. After thawing, incubate the sample at 40°C until all precipitates are dissolved (when stored at low temperatures, urine tends to form precipitates).

Proceed to step 2 (pre-lysis) of the standard protocol (see section 8.1).

9.11 VIRAL DNA FROM URINE

This protocol is designed for purification of viral genomic DNA from urine samples.

Before you start:

- Make sure Lysis Buffer G3, Wash Buffer GW2, and Proteinase K are prepared (see section 7.1)
- Set an incubator or water bath to 56°C.
- Preheat Elution Buffer G to 70°C.

1 **Sample preparation**

Note: If frozen urine samples are used, precipitates may appear after thawing and must be dissolved before centrifugation. Incubate sample at 37-40°C for 30 min. If precipitates do not dissolve, allow precipitate to sediment and perform this step with only the supernatant.

Centrifuge aliquots of the urine sample for 10 min at full speed (e.g., 4ml: 4 x 1ml in a 1.5ml microcentrifuge tube).

Carefully decant the supernatant.

2 **Pre-lysis**

Resuspend the pellet in Lysis Buffer GL and Proteinase K.

Follow either procedure A or B:

A) Resuspend first pellet in 180µl Lysis Buffer GL and 25µl Proteinase K. Transfer the resuspended solution from tube 1 to the tube containing the pellet from the second 1ml aliquot. Resuspend this pellet. Repeat for the pellets from aliquots 3 and 4. The tube should now contain a resuspensed solution of all four pellets.

Proceed with Step 3.

Or

B) Resuspend each pellet individually with 180µl Lysis Buffer GL and 25µl Proteinase K. Pool all four resuspended pellets together into one tube. Proceed to step 3.

Note: With this procedure, at step 5, the ISOLATE II Genomic DNA Spin Column will need to be loaded/centrifuged in successive steps, due to the increased lysate volume.

3 **Lyse sample**

Add 200µl Lysis Buffer G3, vortex and incubate for a minimum of 20 min at 70°C.

4 **Adjust DNA binding conditions**

Add 210µl ethanol (96-100%) to sample and vortex vigorously.

5 **Bind DNA**

Apply sample to an ISOLATE II Genomic DNA Spin Column placed in a Collection Tube.

Centrifuge 1 min at 4,500 x g.

Discard flow-through and return the column to Collection Tube.

6 **Wash silica membrane**

Add 500µl Wash Buffer GW1 to the column. Centrifuge for 1 min at 4,500 x g. Discard flow-through and return the column to Collection Tube.

Add 600µl Wash Buffer GW2 to the column. Centrifuge for 2 min at 11,000 x g. Discard the flow-through and return the column to the Collection Tube.

7 **Dry silica membrane**

Open the lid of the ISOLATE II Genomic DNA Spin Column and incubate for 1-2 min at 70°C.

Residual ethanol is removed during this step.

8 **Elute DNA**

Add 70µl pre-warmed (70°C) Elution Buffer G to the column. Close the lid and incubate for an additional 3-5 min at 70°C. Centrifuge for 1 min at 4,500 x g.

9.12 BACTERIAL DNA FROM CULTURES, CLINICAL SPECIMENS OR BIOLOGICAL FLUIDS

This protocol is suited for the detection of bacterial genomic DNA from a range of starting materials.

Before you start:

- Make sure Lysis Buffer G3, Wash Buffer GW2, and Proteinase K are prepared (see section 7.1)
- Set an incubator or water bath to 56°C.
- Preheat Elution Buffer G to 70°C.

A **Bacterial cultures**

Collect bacteria using an inoculation loop.

Pellet bacteria by centrifugation for 5 min at 13,000 x g and remove the supernatant.

Proceed with step 2 (pre-lysis) of the standard protocol (see section 8.1).

B **Clinical specimens (nasal, pharyngeal, eye or other swabs)**
Collect swab samples and place in 2ml PBS (supplemented with an appropriate fungicide).
Incubate for 2-3 hours at room temperature.
Pellet bacterial cells by centrifugation for 5 min at 13,000 x g.
Proceed with step 2 (pre-lysis) of the standard protocol (see section 8.1).

C **Biological fluids**
Pellet bacteria by centrifugation for 5 min at 13,000 x g and remove the supernatant.
Proceed with step 2 (pre-lysis) of the standard protocol (see section 8.1).

9.13 DETECTION OF *MYCOBACTERIUM TUBERCULOSIS* OR *LEGIONELLA PNEUMOPHILA* IN RESPIRATORY SAMPLES

This protocol is suited for the detection of genomic DNA from *M. tuberculosis* or *L. pneumophila* in respiratory samples (sputum or bronchoalveolar lavage).
Before you start:

- Make sure Lysis Buffer G3, Wash Buffer GW2, and Proteinase K are prepared (see section 7.1)
- Set an incubator or water bath to 56°C.
- Preheat Elution Buffer G to 70°C.
- Prepare N-acetylcysteine/NaOH (2g NaOH; 1.45g sodium citrate; 0.5g N-acetylcysteine. Add water to 100ml).

1 **Sample preparation**
Add 200-500µl sputum or bronchoalveolar lavage to an equal volume N-acetylcysteine/NaOH. Mix by gently vortexing.
Incubate mixture for 25 min at room temperature with shaking.
Adjust volume to 25ml with sterile water.
Centrifuge for 30 min at 4,000 x g. Discard supernatant.
Resuspend pellet in 0.5-1ml Lysis Buffer GL (depending on sample viscosity).
Transfer 200µl of resuspended sample to a new 1.5ml microcentrifuge tube (not provided).
Proceed to step 2 (pre-lysis) of the standard protocol (see section 8.1).

9.14 DETECTION OF *ENTEROHEMORRHAGIC E. COLI* IN FOOD

This protocol is suited for the selective enrichment and isolation of genomic DNA from *enterohemorrhagic E. coli* (EHEC) including serotype 0157:H7 in foodstuffs such as fresh cow's milk.
Before you start:

- Make sure Lysis Buffer G3, Wash Buffer GW2, and Proteinase K are prepared (see section 7.1)
- Prepare Modified Tryptic Soy Broth (mTSB) medium: 30g Tryptic Soy Broth (Gibco), 1.5g bile salts No. 3 (Oxoid), 1.5g KH2PO4. Add 900ml water. Filter the medium and adjust to pH 7.4 with 2M NaOH. Add water to 1L. Autoclave at 121°C for 15 min.
- Set an incubator or water bath to 56°C.
- Preheat Elution Buffer G to 70°C.
- Make a 3.2M solution of sodium acetate.

1 **Sample preparation**
Add 225ml prewarmed (37°C) mTSB medium (supplemented with Novobiocin) and 25ml milk in a sterile 1L flask. Incubate the mixture in a shaking water bath for 5-6h or overnight at 37°C.
Centrifuge 100ml culture for 40 min at 6,000 x g. Decant the supernatant carefully.
Resuspend the pellet in 2ml sterile water. Centrifuge for 10 min at 10,000 x g.

2 **Pre-lysis**
Resuspend the pellet in 180µl Lysis Buffer GL and add 25µl Proteinase K solution.
Perform the standard protocol from step 3 (lyse sample) (see section 8.1).

3 **Post-elution**
After elution, perform an ethanol precipitation of the DNA as follows:
Add 20µl 3.2M sodium acetate and 400µl 96-100% ethanol to 200µl eluate.
Centrifuge for 30 min at 11,000 x g.
Discard the supernatant and wash the pellet with 1ml 70% ethanol.
Resuspend the pellet in 10µl sterile water.

9.15 GENOMIC DNA FROM DENTAL SWABS

Before you start:

- Make sure Lysis Buffer G3, Wash Buffer GW2, and Proteinase K are prepared (see section 7.1)
- Set an incubator or water bath to 56°C.
- Preheat Elution Buffer G to 70°C.

1 **Sample preparation**
Place swab material (e.g. foam, cotton, paper, brushes, plastic) in a 1.5ml microcentrifuge tube (not provided).

2 **Pre-lysis**

Add 180µl Lysis Buffer GL and 25µl Proteinase K to each sample. Close the microcentrifuge tube lid and spin briefly for 15s at 1,500 x g in order to submerge swab material. Incubate at room temperature for 5 min. Vortex the tube vigorously for 15 s and spin briefly for 15s at 1,500 x g.

Incubate the tubes at 70°C in an incubator for 10 min. Place a weight on top of the tubes to stop the caps from opening. Increase the temperature to 95°C for 5 min. Spin briefly for 15s at 1,500 x g to collect any sample from the lids. Open the microcentrifuge tubes.

Depending on the bacterial strains that are to be detected, the incubation at 95°C can be omitted.

2a **Separate lysis solution from dental swabs**

Place an ISOLATE II Filter (not provided) into a Collection Tube.

Cut off the shaft of the dental swab. Transfer the dental swab tip and any associated fluid onto the ISOLATE II Filter.

Centrifuge for 1 min at 11,000 x g. Discard the ISOLATE II Filter and swab.

Transfer as much as possible of the lysate solution to a 1.5ml microcentrifuge tube (not provided).

Proceed to step 3 of the standard protocol (see section 8.1).

9.16 GENOMIC DNA FROM BUCCAL SWABS

Before you start:

- Make sure Lysis Buffer G3, Wash Buffer GW2, and Proteinase K are prepared (see section 7.1)
- Set an incubator or water bath to 56°C.
- Preheat Elution Buffer G to 70°C.

1 **Sample preparation**

Collect samples with cotton, dacron®, or CEP™ swabs (ensure that food/drink is not consumed for at least 30 min prior to sample collection). Scrape firmly against the inside of each cheek several times and allow the swabs to air dry.

2 **Pre-lysis**

Place dry swab material in 2ml microcentrifuge tubes (not provided). Add 400-600µl PBS and 25µl Proteinase K solution to each swab.

The volume of PBS is dependent on the type of swab used (Cotton/Dacron: 400µl; CEP: 600µl).

Mix by vortexing for 5s, repeat, and incubate for 10 min at 56°C.

2a **Separate lysis solution from buccal swabs**

Alternative I

Place an ISOLATE II Filter (not provided) into a Collection Tube. Cut off the shaft of the buccal swab. Transfer the buccal swab tip and any associated fluid onto the ISOLATE II Filter. Centrifuge for 1 min at 11,000 x g. Discard ISOLATE II Filter and swab.

Or:

Alternative II

Transfer as much of the lysate solution as possible into a 1.5ml microcentrifuge tube (not provided). Discard swab and continue with the recovered solution.

3 **Lyse sample**

Add one volume of Lysis Buffer G3 (400 or 600 µl; this is dependent on the swab type and volume of PBS buffer used) and vortex vigorously. Incubate the samples at 70°C for 10 min.

Note: Depending on the number of preparations, additional Lysis Buffer G3 may be required.

4 **Adjust DNA binding conditions**

Add one volume 96–100% ethanol (400 or 600µl, depending on swab type) to each sample and mix by vortexing.

5 **Bind DNA**

Transfer 600µl of the samples into individual ISOLATE II Genomic DNA Spin Columns. Centrifuge at 11,000 x g for 1 min. If the samples have not transferred through the column matrix completely, repeat centrifugation. Discard flow-through.

Place columns back into Collection Tubes and repeat centrifugation with any remaining lysate.

When the entire lysate has been applied, proceed to step 6 (wash silica membrane) of the standard protocol (section 8.1).

9.17 GENOMIC DNA FROM INSECTS

Before you start:

- Make sure Lysis Buffer G3, Wash Buffer GW2, and Proteinase K are prepared (see section 7.1)
- Set an incubator or water bath to 56°C.
- Preheat Elution Buffer G to 70°C.

1 **Sample preparation**

Homogenize a maximum of 50mg insect material under liquid nitrogen. Transfer powdered material into a 1.5ml microcentrifuge tube (not provided).

Proceed to step 2 (pre-lysis) of the standard protocol (see section 8.1).

9.18 GENOMIC DNA FROM SEMEN

To obtain optimal results, a differential extraction method is required in order to separate spermatozoa from other cell types such as epithelial cells and/or blood.

Additional reagents needed:

- Buffer GuEX (200ml)
  - o Mix 2ml sterile 5M Guanidine hydrochloride solution (should not be autoclaved), 2.1ml 1M Tris-Cl (pH 8) solution, 1.05ml 2M NaCl solution, 4.2ml 0.5M EDTA solution and 0.2ml 1M NaOH solution
  - o Add water to a volume of 200ml. The pH should be between 8–8.5
- Isopropanol

Before you start:

- Prepare buffer GuEX
- Make sure Lysis Buffer G3, Wash Buffer G2 and Proteinase K are prepared (see section 7.1)
- Preheat Elution Buffer G to 70°C

1 **Sample preparation**
Transfer sample to a 1.5ml microcentrifuge tube. Add 950µl buffer GuEX and 50µl Proteinase K solution. Incubate no longer than 15 min at 37 °C.

2 **Separate sample**
Centrifuge mixture for 4 min at 12,000 x g at room temperature. The pellet contains sperm cells (sample A pellet). Free DNA (from epithelial cells and leukocytes) is in the supernatant (sample B supernatant).

3 **Remove supernatant**
Carefully remove the supernatant (sample B supernatant). Transfer to a fresh tube and process separately (see step 6).

4 **Add buffer to pellet**
Add 700µl buffer GuEX to the pellet (sample A pellet), centrifuge for 4 min at 12,000 x g, and discard the supernatant. Repeat this wash step 2–3 times.

5 **Resuspend pellet**
Resuspend sample A pellet in a minimum of 300µl Buffer GL.

6 **Lyse sample**
- o Sample A pellet: Add 25µl Proteinase K stock solution, mix by vortexing and incubate overnight at 60–65 °C.
- o Sample B supernatant: Add 10µl Proteinase K stock solution, mix by vortexing and incubate overnight at 60–65 °C.

7 **Clarify sample**
Centrifuge samples for 5 min at 12,000 x g at room temperature (RT) in order to remove any unsoluble cell material. Proceed with the clear supernatant.

8 **Bind DNA**
- o Sample A pellet: Add 300µl Lysis Buffer G3 and 300µl isopropanol to the clear supernatant and apply the sample successively to the ISOLATE II Genomic DNA Spin Column. Centrifuge 1 min at 6,000 x g (RT). If the sample is not passing through the membrane completely, repeat centrifugation step.
- o Sample B supernatant: Add 400µl of isopropanol to the clear supernatant and apply the sample successively to the ISOLATE II Genomic DNA Spin Column. Centrifuge 1 min at 6,000 x g (RT). If the sample is not passing through the membrane completely, repeat centrifugation step.

9 **Wash silica membrane**
Add 500µl Wash Buffer GW2 (including ethanol) to the spin column and centrifuge 1 min at 6,000 x g (RT). Discard the flow-through. Repeat this wash step and discard flow-through.

10 **Dry silica membrane**
Centrifuge 2 min at 6,000 x g (RT) to remove Wash Buffer GW2 completely.

11 **Elute DNA**
Place the ISOLATE II Genomic DNA Spin Column in a clean 1.5ml centrifuge tube and elute the DNA with 100–200µl preheated Elution Buffer G (70° C). After 2 min incubation, centrifuge for 1 min at 6,000 x g (RT).

10. TROUBLESHOOTING GUIDE

LOW DNA YIELD	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Incomplete cell lysis	Sample must be vortexed vigorously immediately after addition of Lysis Buffer GL / Proteinase K solution. Proteinase K digestion not optimal: never add Proteinase K directly to Lysis Buffer GL. Store dissolved Proteinase K at-20°C for up to 6 months.
Reagents not applied correctly	Prepare buffers and Proteinase K solution according to instructions (section 7.1). Reagents not stored optimally: store Proteinase K solution at-20°C. Store all other components at room temperature. Keep bottles tightly closed to prevent evaporation or contamination.
Suboptimal elution from the column	Apply preheated (70°C) Elution Buffer G directly onto the center of the silica membrane. If not using Elution Buffer G, make sure elution buffer used is slightly alkaline (pH 8.5). For high yields from large amounts of material, we recommend elution with 200µl Elution Buffer G and incubation of the closed columns in an incubator at 70°C for 5 min before centrifugation.
POOR DNA QUALITY	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Incomplete cell lysis	Sample must be vortexed vigorously immediately after addition of Lysis Buffer GL / Proteinase K solution. Decreased Proteinase K activity: store dissolved Proteinase K at-20°C for 6 months.
Reagents not applied correctly	Prepare buffers and Proteinase K solution according to instructions (section 7.1). Make sure ethanol is added to lysates before loading on columns.
RNA in sample	To remove RNA add 20µl RNase A solution (20mg/ml) (not included) before addition of lysis buffer.
CLOGGED COLUMNS	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Too much sample material	Do not use more sample material than recommended in protocol. If insoluble material remains in the lysate, spin down the debris and transfer the clear supernatant to a new tube before proceeding with addition of Lysis Buffer G3 and ethanol.
Incomplete lysis	Sample must be vortexed vigorously immediately after addition of Lysis Buffer GL / Proteinase K solution. Decreased Proteinase K activity: store dissolved Proteinase K at-20°C for up to 6 months.
Reagents not applied correctly	Prepare buffers and Proteinase K solution according to instructions (section 7.1). Ensure ethanol is added to lysates before loading on columns.
SUBOPTIMAL PERFORMANCE OF EXTRACTED GENOMIC DNA IN ENZYMATIC REACTIONS	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Ethanol carry-over	Be sure to remove all traces of Wash Buffer GW2 before eluting the DNA. If necessary repeat silica membrane drying step a second time. Do not chill Wash Buffer GW2 before use. Cold buffer will not remove salt effectively. Equilibrate Wash Buffer GW2 to room temperature before use.
Contamination of DNA with inhibitory substances	We recommend elution with Elution Buffer G, as chemicals such as EDTA that are found in other buffers can interfere with downstream applications. If the A_{260}/A_{280} ratio of the eluate is below 1.6, repeat the purification procedure: Add equal volumes of Lysis Buffer G3 and ethanol to the eluate, load column and proceed with step 3 of the protocol.

A. TECHNICAL SUPPORT

For technical assistance or more information on these products, please email us at tech@bioline.com

B. ORDERING INFORMATION

PRODUCT	PACK SIZE	CAT NO.
ISOLATE II Genomic DNA Kit	10 Preps	BIO-52065
ISOLATE II Genomic DNA Kit	50 Preps	BIO-52066
ISOLATE II Genomic DNA Kit	250 Preps	BIO-52067

C. ASSOCIATED PRODUCTS

PRODUCT	PACK SIZE	CAT NO.
ISOLATE II Blood DNA Kit	50 Preps	BIO-52063
ISOLATE II Plant DNA Kit	50 Preps	BIO-52069
MyTaq™ HS DNA Polymerase	250 Units	BIO-21111
SensiFAST™ SYBR No-ROX Kit	200 Reactions	BIO-98002

D. PRODUCT WARRANTY AND DISCLAIMER

Bioline warrants that its products will conform to the standards stated in its product specification sheets in effect at the time of shipment. Bioline will replace free of charge any product that does not conform to the specifications. This warranty limits Bioline's liability only to the replacement of the product.

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