

ISOLATE II Genomic DNA Kit

Product Manual



A Meridian Life Science® Company

**ISOLATE II** Genomic DNA Kit**ISOLATE II Genomic DNA Kit**

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1. KIT CONTENTS

COMPONENT	10 Preps	50 Preps	250 Preps
ISOLATE II Genomic DNA Spin Columns (green)	10	50	250
Collection Tubes (2 mL)	20	100	500
Lysis Buffer GL	5 mL	20 mL	100 mL
Buffer G3	10 mL	15 mL	75 mL
Wash Buffer GW1	6 mL	30 mL	150 mL
Wash Buffer GW2 [†] (concentrate)	6 mL	12 mL	50 mL
Elution Buffer G	13 mL	13 mL	60 mL
Proteinase K (lyophilized)	6 mg	30 mg	2 x 75 mg
Proteinase K Buffer PR	1.8 mL	1.8 mL	8 mL
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 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 G3 and Wash Buffer GW1 contain guanidine hydrochloride. This chemical is harmful when in skin contact, inhaled or ingested.

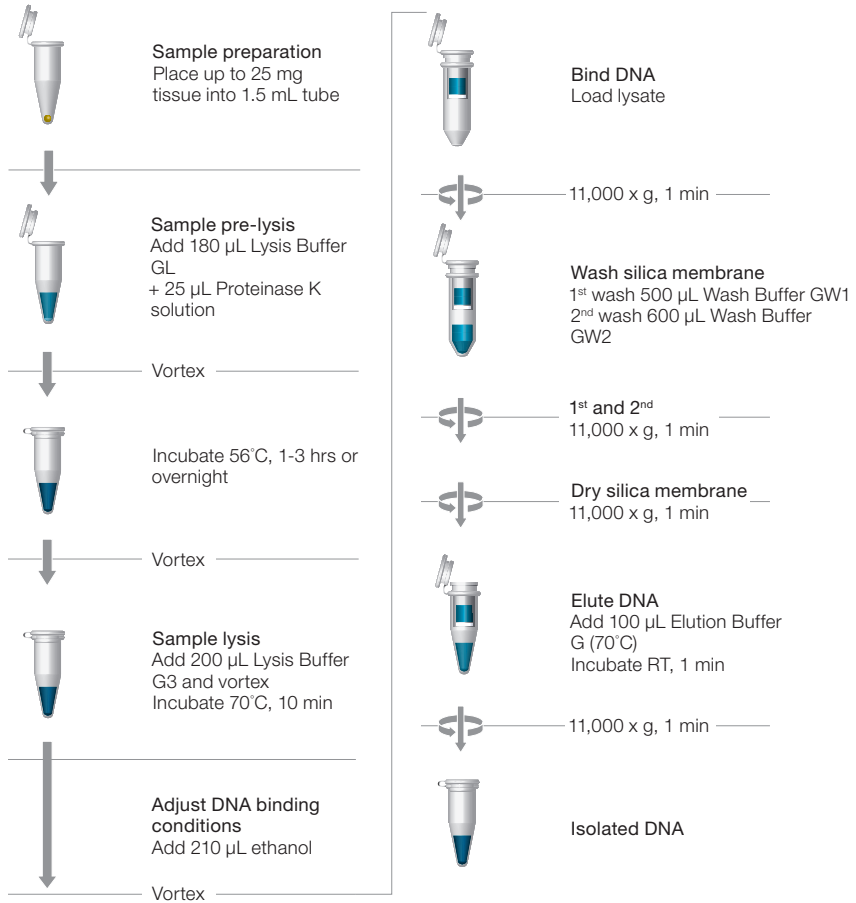
For detailed information, please consult the material data safety sheets (MSDSs) available on our website at www.bioline.com.

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 20-35 μg (see below).

ISOLATE II GENOMIC DNA COLUMN SPECIFICATIONS	
Max. binding capacity	60 μg DNA
A_{260}/A_{280}	1.7-1.9
Typical yield	20-35 μg
Elution volume	60-100 μL
Sample material	
Tissue	1-25 mg
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[†]
- Microcentrifuge tubes (1.5 mL)
- 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 Wash Buffer GW2

Add 96-100% ethanol to Wash Buffer GW2 Concentrate: 24 mL for the 10 prep kit, 48 mL for the 50 prep kit and 200 mL 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: 260 μ L for the 10 prep kit, 1.35 mL for the 50 prep kit and 3.35 mL 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 100 μ L Elution Buffer G (to increase yield to 90-100%).
- High concentration: One elution step with 60 μ L Elution Buffer G (to increase concentration by about 130%). Maximal yield 80%.
- High yield and high concentration: Two elution steps. Add 50 μ L Elution Buffer G, incubate for 3 min and centrifuge, repeat with a second 50 μ L 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 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 25 mg of tissue into small pieces. Place the sample in a 1.5 mL 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 25 mg of tissue to a 1.5 mL microcentrifuge tube (not supplied), add 50-75 µL PBS (not supplied) and homogenize.

1.2 Cultured cells

Resuspend up to 10⁷ cells in a final volume of 200 µL Lysis Buffer GL. Add 25 µL Proteinase K solution and 200 µL Lysis Buffer G3. Incubate the sample at 70°C for 10-15 min (proceed to step 4).

2 Pre-lysis

Add 180 µL Lysis Buffer GL and 25 µL 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 200 µL 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 210 µL 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 500 μL Wash Buffer GW1. Centrifuge for 1 min at 11,000 x g. Discard flow-through and reuse Collection Tube.
- Add 600 μL 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 1.5 mL microcentrifuge tube (not supplied).

8 Elute DNA

Add 100 μL 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 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 0.6 cm pieces of mouse tail and place in a 1.5 mL centrifuge tube (not supplied).

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

2 Pre-lysis

Add 180 μL Lysis Buffer GL and 25 μL 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 200 μL supernatant to a new centrifuge tube.

3 Lyse sample

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

4 Adjust DNA binding conditions

Add 210 µL 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 Wash Buffer GW2 and Proteinase K are prepared (see section 7.1).
- For hard-to-lyse bacteria make up the following lysis buffer (20 mM Tris/HCl; 2 mM EDTA; 1% Triton X-100; pH 8, supplemented with 20 mg/mL lysozyme or 0.2 mg/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 1 mL of bacterial culture can be used for the preparation depending on density, culture medium, bacterial strain etc.

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

2 Pre-lysis

Resuspend pellet in 180 µL Lysis Buffer GL and 25 µL 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 25 µL 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 Wash Buffer GW2 and Proteinase K are prepared (see section 7.1).
- Make up sorbitol buffer (1.2 M sorbitol; 10 mM CaCl₂; 0.1 M Tris/HCl pH 7.5; 35 mM β-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 3 mL YPD yeast culture ($OD_{600} \leq 10$) for 10 min at 5,000 x g. Remove supernatant.

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

2 Pre-lysis

Resuspend the pellet in 600 μ L 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 180 μ L Lysis Buffer GL and 25 μ L 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 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 30 mm² in area) as accurately as possible. Cut spots into small pieces and place into a 1.5 mL microcentrifuge tube (not supplied).

2 Pre-lysis

Add 180 μ L Lysis Buffer GL and incubate at 94°C for 10 min.

Cool and add 25 μ L 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 200 μ L 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 Wash Buffer GW2 and Proteinase K are prepared (see section 7.1).
- Preheat Elution Buffer G to 70°C.

1 **Lyse blood**

Add 25 µL Proteinase K and 200 µL sample into a 1.5 mL microcentrifuge tube (not supplied).

Note: Make up sample to 200 µL with PBS if using less volume. For cultured cells, resuspend up to 5×10^6 cells in 200 µL PBS.

Add 200 µL 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 210 µL 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 (2 mL).

Proceed with step 6 of the standard protocol (see section 8.1).

9.6 HAIR ROOTS

Before you start:

- Make sure 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 1.5 mL centrifuge tube (not supplied).

2 **Pre-lysis**

Add 180 µL 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 25 µL 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 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 25 mg). With tweezers or toothpicks place the sections into microcentrifuge tubes (not supplied).

Add 1 mL 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 1 mL 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 Wash Buffer GW2 and Proteinase K are prepared (see section 7.1).
- Check that TE (10 mM Tris-HCl; 1 mM EDTA, pH 8.0) is available.
- Set an incubator or water bath to 37°C and 56°C.
- Preheat Elution Buffer G to 70°C.

1 Sample preparation

Add 250 mg fecal material to 1 mL 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 0.2-1 mL Lysis Buffer GL. Add sufficient buffer to thoroughly resuspend the sample.

Transfer 200 µL of the resuspended sample to a new microcentrifuge tube and add 25 µL 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 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 water.

1 Sample preparation

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

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

2 Pre-lysis

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

3 Lyse sample

Add 400 µL 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 420 µL 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 600 µL Wash Buffer GW1 to the column. Centrifuge for 1 min at 4,500 x g. Discard the flow-through and reuse Collection Tube.
- Add 600 µL Wash Buffer GW2 to the column. Centrifuge for 1 min at 4,500 x g. Discard the flow-through and reuse Collection Tube.
- Add 600 µL 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 1.5 mL microcentrifuge tube (not supplied).

8 **Elute DNA**

Add 100 µL 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 10 µL purified DNA as template in a 20 µL PCR reaction.

Add an inhibition control mix (10 µL purified DNA template with human DNA). Amplify with primers specific for a human DNA sequence such as β -actin, β -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 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 1 mL urine sample at 13,000 x g for 30 min. Discard the supernatant.

Add another 1 mL 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 1 mL 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 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., 4 mL: 4 x 1 mL in a 1.5 mL microcentrifuge tube).

Carefully decant the supernatant.

2 **Pre-lysis**

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

3 **Lyse sample**

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

4 **Adjust DNA binding conditions**

Add 210 µL 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 500 µL 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 600 µL 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 70 µL 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 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 2 mL 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 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 (2 g NaOH; 1.45 g sodium citrate; 0.5 g N-acetylcysteine. Add water to 100 mL).

1 Sample preparation

Add 200-500 µL 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 25 mL with sterile water.

Centrifuge for 30 min at 4,000 x g. Discard supernatant.

Resuspend pellet in 0.5-1 mL Lysis Buffer GL (depending on sample viscosity).

Transfer 200 µL of resuspended sample to a new 1.5 mL 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 O157:H7 in foodstuffs such as fresh cow's milk.

Before you start:

- Make sure Wash Buffer GW2 and Proteinase K are prepared (see section 7.1)
- Prepare Modified Tryptic Soy Broth (mTSB) medium: 30 g Tryptic Soy Broth (Gibco), 1.5 g bile salts No.3 (Oxoid), 1.5 g KH₂PO₄. Add 900 mL water. Filter the medium and adjust to pH 7.4 with 2 M NaOH. Add water to 1 L. 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 3.2 M solution of sodium acetate.

1 Sample preparation

Add 225 mL prewarmed (37°C) mTSB medium (supplemented with Novobiocin) and 25 mL milk in a sterile 1 L flask. Incubate the mixture in a shaking water bath for 5-6 hours or overnight at 37°C.

Centrifuge 100 mL culture for 40 min at 6,000 x g. Decant the supernatant carefully.

Resuspend the pellet in 2 mL sterile water. Centrifuge for 10 min at 10,000 x g.

2 Pre-lysis

Resuspend the pellet in 180 µL Lysis Buffer GL and add 25 µL 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 20 µL 3.2 M sodium acetate and 400 µL 96-100% ethanol to 200 µL eluate.

Centrifuge for 30 min at 11,000 x g.

Discard the supernatant and wash the pellet with 1 mL 70% ethanol.

Resuspend the pellet in 10 µL sterile water.



9.15 GENOMIC DNA FROM DENTAL SWABS

Before you start:

- Make sure 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 1.5 mL microcentrifuge tube (not provided).

2 Pre-lysis

Add 180 µL Lysis Buffer GL and 25 µL 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 1.5 mL 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 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.
- Make sure phosphate buffered saline (PBS) is available

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 2 mL microcentrifuge tubes (not provided). Add 400-600 μ L PBS and 25 μ L Proteinase K solution to each swab.

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

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.

Alternative II

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.

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

Alternative III

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

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

3 **Lyse sample**

Add one volume of Lysis Buffer G3 (400 or 600 μ L; 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 600 μ L, depending on swab type) to each sample and mix by vortexing.

5 **Bind DNA**

Transfer 600 μ L 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 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 50 mg insect material under liquid nitrogen. Transfer powdered material into a 1.5 mL 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 (200 mL)
 - Mix 2 mL sterile 5 M Guanidine hydrochloride solution (should not be autoclaved), 2.1 mL 1 M Tris-Cl (pH 8) solution, 1.05 mL 2 M NaCl solution, 4.2 mL 0.5 M EDTA solution and 0.2 mL 1 M NaOH solution
 - Add water to a volume of 200 mL. The pH should be between 8-8.5
- Isopropanol

Before you start:

- Prepare buffer GuEX
- Make sure Proteinase K is prepared (see section 7.1)
- Preheat Elution Buffer G to 70°C

1 Sample preparation

Transfer sample to a 1.5 mL microcentrifuge tube. Add 950 μ L buffer GuEX and 50 μ L 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 700 μ L 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 300 μ L Buffer GL.

6 Lyse sample

- o Sample A pellet: Add 25 μ L Proteinase K stock solution, mix by vortexing and incubate overnight at 60-65 °C.
- o Sample B supernatant: Add 10 μ L 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 insoluble cell material. Proceed with the clear supernatant.

8 Bind DNA

- o Sample A pellet: Add 300 μ L Lysis Buffer G3 and 300 μ L 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 400 μ L 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 500 μ L 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 1.5 ml centrifuge tube and elute the DNA with 100-200 μ L 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 200 µL 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 20 µL RNase A solution (20 mg/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 this product, 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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