# ISOLATE II Genomic DNA Kit

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## General Information

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1. KIT CONTENTS

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<th>10 Preps</th>
<th>50 Preps</th>
<th>250 Preps</th>
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<tr>
<td>ISOLATE II Genomic DNA Spin Columns (green)</td>
<td>10</td>
<td>50</td>
<td>250</td>
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<tr>
<td>Collection Tubes (2 mL)</td>
<td>20</td>
<td>100</td>
<td>500</td>
</tr>
<tr>
<td>Lysis Buffer GL</td>
<td>5 mL</td>
<td>20 mL</td>
<td>100 mL</td>
</tr>
<tr>
<td>Buffer G3</td>
<td>10 mL</td>
<td>15 mL</td>
<td>75 mL</td>
</tr>
<tr>
<td>Wash Buffer GW1</td>
<td>6 mL</td>
<td>30 mL</td>
<td>150 mL</td>
</tr>
<tr>
<td>Wash Buffer GW2† (concentrate)</td>
<td>6 mL</td>
<td>12 mL</td>
<td>50 mL</td>
</tr>
<tr>
<td>Elution Buffer G</td>
<td>13 mL</td>
<td>13 mL</td>
<td>60 mL</td>
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<tr>
<td>Proteinase K (lyophilized)</td>
<td>6 mg</td>
<td>30 mg</td>
<td>2 x 75 mg</td>
</tr>
<tr>
<td>Proteinase K Buffer PR</td>
<td>1.8 mL</td>
<td>1.8 mL</td>
<td>8 mL</td>
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† 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).

<table>
<thead>
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<th>ISOLATE II GENOMIC DNA COLUMN SPECIFICATIONS</th>
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<tr>
<td>Max. binding capacity</td>
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<tr>
<td>$A_{260}/A_{280}$</td>
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<td>Typical yield</td>
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Genomic DNA Isolation

Sample preparation
Place up to 25 mg tissue into 1.5 mL tube

Sample pre-lysis
Add 180 μL Lysis Buffer GL + 25 μL Proteinase K solution
Vortex
Incubate 56°C, 1-3 hrs or overnight
Vortex

Sample lysis
Add 200 μL Lysis Buffer G3 and vortex
Incubate 70°C, 10 min

Adjust DNA binding conditions
Add 210 μL ethanol
Vortex

Bind DNA
Load lysate

11,000 x g, 1 min

Wash silica membrane
1st wash 500 μL Wash Buffer GW1
2nd wash 600 μL Wash Buffer GW2
1st and 2nd washes
11,000 x g, 1 min

Dry silica membrane
11,000 x g, 1 min

Elute DNA
Add 100 μL Elution Buffer G (70°C)
Incubate RT, 1 min

11,000 x g, 1 min

Isolated DNA
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 μL 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 μL Elution Buffer G (to increase yield to 90-100%).
- High concentration: One elution step with 60 μL Elution Buffer G (to increase concentration by about 130%). Maximal yield 80%.
- High yield and high concentration: Two elution steps. Add 50 μL Elution Buffer G, incubate for 3 min and centrifuge, repeat with a second 50 μL 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 10^7 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 CaCl$_2$; 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$_{600}$ ≤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 μL 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 μL Lysis Buffer GL and 25 μL 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 mm$^2$ 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 μL Lysis Buffer GL and incubate at 94°C for 10 min.
Cool and add 25 μL 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 μL 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 x 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. The 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 0157: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 μL PBS and 25 μL Proteinase K solution to each swab.

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

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 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 600 μL, depending on swab type) to each sample and mix by vortexing.

5 **Bind DNA**

Transfer 600 μL 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 μL buffer GuEX and 50 μL 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 μL 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 μL Buffer GL.

6 **Lyse sample**
- Sample A pellet: Add 25 μL Proteinase K stock solution, mix by vortexing and incubate overnight at 60‑65 °C.
- Sample B supernatant: Add 10 μL 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**
- Sample A pellet: Add 300 μL Lysis Buffer G3 and 300 μL 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.
- Sample B supernatant: Add 400 μL 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 μL 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 μL 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

<table>
<thead>
<tr>
<th>POSSIBLE CAUSE</th>
<th>RECOMMENDED SOLUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incomplete cell lysis</td>
<td>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.</td>
</tr>
<tr>
<td>Reagents not applied correctly</td>
<td>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.</td>
</tr>
<tr>
<td>Suboptimal elution from the column</td>
<td>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.</td>
</tr>
</tbody>
</table>

### POOR DNA QUALITY

<table>
<thead>
<tr>
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<th>RECOMMENDED SOLUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incomplete cell lysis</td>
<td>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.</td>
</tr>
<tr>
<td>Reagents not applied correctly</td>
<td>Prepare buffers and Proteinase K solution according to instructions (section 7.1). Make sure ethanol is added to lysates before loading on columns.</td>
</tr>
<tr>
<td>RNA in sample</td>
<td>To remove RNA add 20 μL RNase A solution (20 mg/mL) (not included) before addition of lysis buffer.</td>
</tr>
</tbody>
</table>

### CLOGGED COLUMNS

<table>
<thead>
<tr>
<th>POSSIBLE CAUSE</th>
<th>RECOMMENDED SOLUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Too much sample material</td>
<td>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.</td>
</tr>
</tbody>
</table>

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[23] Genomic DNA Kit
<table>
<thead>
<tr>
<th>Incomplete lysis</th>
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</tr>
</tbody>
</table>

**SUBOPTIMAL PERFORMANCE OF EXTRACTED GENOMIC DNA IN ENZYMATIC REACTIONS**

<table>
<thead>
<tr>
<th>POSSIBLE CAUSE</th>
<th>RECOMMENDED SOLUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol carry-over</td>
<td>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.</td>
</tr>
<tr>
<td>Contamination of DNA with inhibitory substances</td>
<td>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.</td>
</tr>
</tbody>
</table>
A. TECHNICAL SUPPORT
For technical assistance or more information on this product, please email us at tech@bioline.com

B. ORDERING INFORMATION

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>PACK SIZE</th>
<th>CAT NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISOLATE II Genomic DNA Kit</td>
<td>10 Preps</td>
<td>BIO-52065</td>
</tr>
<tr>
<td>ISOLATE II Genomic DNA Kit</td>
<td>50 Preps</td>
<td>BIO-52066</td>
</tr>
<tr>
<td>ISOLATE II Genomic DNA Kit</td>
<td>250 Preps</td>
<td>BIO-52067</td>
</tr>
</tbody>
</table>

C. ASSOCIATED PRODUCTS

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>PACK SIZE</th>
<th>CAT NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISOLATE II Blood DNA Kit</td>
<td>50 Preps</td>
<td>BIO-52063</td>
</tr>
<tr>
<td>ISOLATE II Plant DNA Kit</td>
<td>50 Preps</td>
<td>BIO-52069</td>
</tr>
<tr>
<td>MyTaq™ HS DNA Polymerase</td>
<td>250 Units</td>
<td>BIO-21111</td>
</tr>
<tr>
<td>SensiFAST™ SYBR No-ROX Kit</td>
<td>200 Reactions</td>
<td>BIO-98002</td>
</tr>
</tbody>
</table>

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.