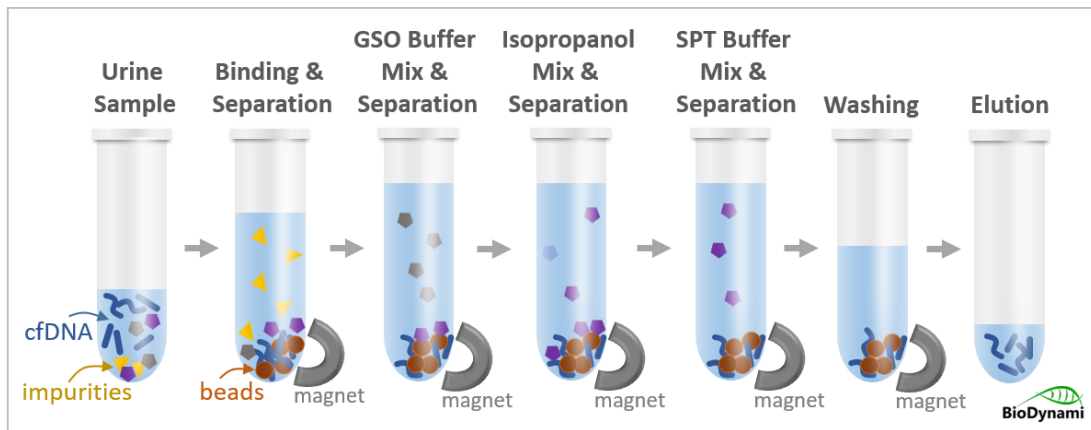


Urine Cell-free DNA Extraction Kit (Magnetic Beads)

Catalog No.	50103S	50103L	50104S	50104L
Rxns	10 rxns	40 rxns	10 rxns	40 rxns
Sample volume	5 ml	5 ml	40 ml	40 ml

Description

The **Urine Cell-free DNA Extraction Kit (Magnetic Beads)** is developed for the efficient extraction of cell-free DNA (cfDNA) from fresh or frozen urine samples. Urinary cfDNA carries valuable genetic and epigenetic information, making it a powerful tool for biomarker discovery in cancer and other disease research.



Urine offers two key advantages as a source for biomarker discovery: First, larger sample volumes can be obtained compared to plasma or serum. Second, urine collection is truly non-invasive. Additionally, the same individual can provide repeated samples over time, enabling longitudinal studies.

Urinary cfDNA has been increasingly studied for the diagnosis and prognosis of various cancers and diseases. However, sample composition can vary significantly between individuals due to factors such as cellular heterogeneity, health and disease status, stress levels, medical treatments, and more.

DNA in urine is primarily derived from cells shed from the urinary tract. During apoptosis and necrosis, chromosomal DNA is fragmented—a process that is more pronounced under pathological conditions including cancer development, inflammation, and immune responses. As a result, urine cfDNA is typically highly degraded, with fragments around 100 bp or shorter.

Our kit leverages Solid Phase Reversible Immobilization (SPRI) technology—a simple, bead-based method ideally suited for recovering cfDNA from both fresh and frozen urine samples. The protocol eliminates tedious steps such as spin column centrifugation and vacuum filtration, offering a reliable and convenient workflow for isolating high-quality cfDNA.

Recovered DNA fragments can be eluted in volumes as low as 10 μ l, enabling high-concentration yields for sensitive applications. The extracted cfDNA is fully compatible with a wide range of downstream techniques, including bisulfite sequencing, next-generation sequencing (NGS), microarray, PCR, qPCR, and more.

Two kit formats are available to accommodate different urine input volumes: 5 ml (Cat. # 50103) and 40 ml (Cat. # 50104).

Specifications	
Sample type	Fresh or frozen urine samples
Sample volume	5 ml (Cat. # 50103) or 40 ml (Cat. # 50104)
Technology	Magnetic beads
Purity	cfDNA is NGS and PCR ready. Typical A260/A280 is around 1.8. Note: sample quantification or purity assessment using a spectrophotometer is not recommended due to the limited amount of DNA.
Yield range	Typical yield: 0.5 ng/ml to 20 ng/ml from healthy donors
Fragment sizes	From 60 bp to >10 kb genomic DNA
Elution volume	As low as 10 μ l is OK

Component

Catalog No.	50103S	50103L	50104S	50104L
US Buffer	-	-	0.5 ml	2 ml
THP Buffer	1 ml	4 ml	4 ml	16 ml
Urine Lysis Buffer	40 ml	160 ml	3 ml	12 ml
Urine-A Beads	50 ml	200 ml	-	-
Urine-B Beads	-	-	4 ml	16 ml
GSO Buffer	40 ml	160 ml	6 ml	24 ml
Proteinase K	0.2 ml	0.8 ml	0.1 ml	0.4 ml
SPT Buffer	3 ml	12 ml	3 ml	12 ml
Elution Buffer	0.44 ml	1.8 ml	0.44 ml	1.8 ml

Storage Condition

- Store Urine-A Beads or Urine-B Beads, Proteinase K, and SPT Buffer at 4°C, stable up to 12 months.
- Store Urine Lysis Buffer, GSO Buffer, THP Buffer, US Buffer, and Elution Buffer at room temperature, stable up to 12 months.

Reagent & Equipment Needed (not provided in this reagent)

- Magnetic particle concentrator
- Vortexer
- Rotator
- Heat block, incubator, or water bath
- 96-well plates or 96-deep well plates, microfuge tubes
- 80% isopropanol (prepare before use) and 100% isopropanol
- 80% ethanol (prepare before use)

Protocol A (Cat. # 50103S and 50103L for 5 ml urine samples)

Note: Invert or shake the beads bottles thoroughly to resuspend the Urine Beads.

- 1) Mix 5 ml of urine sample with 4 ml of **Urine Lysis Buffer**, 5 ml of **Urine-A Beads**, and 20 μ l of **Proteinase K** in 15 ml tube. Rotate on a rotator to mix for 5 min.
- 2) Incubate at 56°C for 45 min. Mix by inverting the tubes several times every 10 min.
- 3) Put the sample on a magnet for 15 min, and completely remove the supernatant slowly with a pipette. **Note:** Fast removal of supernatant may cause both beads loss and solution retention on the tube wall, which will decrease DNA amount and quality.
- 4) Add 4 ml of **GSO Buffer**, screw the cap tightly, put the tube upside down, and vortex briefly. Turn the tube upright and vortex briefly.
- 5) Load the sample on a magnet, incubate for 5 min, and completely remove the supernatant slowly with a pipette.
- 6) Add 1 ml of **80% isopropanol**, vortex the tube, and transfer the supernatant to a 1.5 ml tube.
- 7) Load the sample on a magnet, incubate for 1 min, and discard the supernatant.
- 8) Add 100 μ l of **THP Buffer**, vortex the tube, and incubate for 1 min.
- 9) Add 300 μ l of **SPT Buffer**, vortex the tube, and incubate for 15 min. **Note:** Slow pipetting of the viscous SPT Buffer is needed for precise aliquots.
- 10) Load the sample on a magnet, incubate for 5 min, and completely remove the supernatant slowly with a pipette.
- 11) Add 800 μ l of **80% ethanol** and incubate for 2 min. Discard the supernatant without disturbing the beads. Repeat this step one more time.
- 12) Air-dry the beads on the magnet for 10 min. Remove ethanol completely with a pipette. **Note:** Do not over-dry the beads. Go to step 13 immediately if beads cracks are observed.
- 13) Remove the tubes from the magnet and resuspend the beads in **Elution Buffer** (Tris-HCl 10 mM, pH 8.0), low TE buffer, or TE buffer. **Note:** Resuspending the beads in less than 10 μ l may reduce the yield.
- 14) Load the plate on the magnet, incubate for 1 min, and transfer the supernatant (containing sample) to a new plate or tubes without disturbing the beads.

cfDNA concentration and sizing

Determine cfDNA concentration by BioDynami dsDNA Quantification High Sensitivity Kits (Cat. # 40042 for Qubit fluorometer; Cat. # 40540H for microplate reader). Sizing of both cfDNA and genomic DNA fractions can be determined on Agilent TapeStation cfDNA tape kits. If genomic DNA is not required to be measured, cfDNA size can also be determined on HS DNA 1000 tape kits. cfDNA is now ready for downstream applications.

Protocol B (Cat. # 50104S and 50104L for 40 ml urine samples)

Note: Invert or shake the beads bottles thoroughly to resuspend the Urine Beads.

- 1) Add 50 μ l of **US Buffer** and 6.5 ml of **isopropanol** to 40 ml of urine sample, mix and centrifuge at 3,000 g for 15 min. Discard the solution carefully without disturbing the pellet.
- 2) Resuspend the pellet in 300 μ l of **THP Buffer** and transfer to a 1.5 ml tube.
- 3) Add 300 μ l of **Urine Lysis Buffer**, 400 μ l of **Urine-B Beads**, and 10 μ l of **Proteinase K**. Mix by vortexing, and incubate at 56°C for 30 min.
- 4) Put the sample on a magnet for 10 min, and completely remove the supernatant slowly with a pipette. **Note:** Fast removal of supernatant may cause both beads loss and solution retention on the tube wall, which will decrease DNA amount and quality.
- 5) Add 600 μ l of **GSO Buffer**, and vortex briefly to resuspend the beads.
- 6) Load the sample on a magnet, incubate for 5 min, and completely remove the supernatant slowly with a pipette.
- 7) Add 1 ml of **80% isopropanol**, vortex the tube, and transfer the supernatant to a 1.5 ml tube.
- 8) Load the sample on a magnet, incubate for 1 min, and discard the supernatant.
- 9) Add 100 μ l of **THP Buffer**, vortex the tube, and incubate for 1 min.
- 10) Add 300 μ l of **SPT Buffer**, vortex the tube, and incubate for 15 min. **Note:** Slow pipetting of the viscous SPT Buffer is needed for precise aliquots.
- 11) Load the sample on a magnet, incubate for 5 min, and completely remove the supernatant slowly with a pipette.
- 12) Add 800 μ l of **80% ethanol** and incubate for 2 min. Discard the supernatant without disturbing the beads. Repeat this step one more time.
- 13) Air-dry the beads on the magnet for 10 min. Remove ethanol completely with a pipette. **Note:** Do not over-dry the beads. Go to step 14 immediately if beads cracks are observed.
- 14) Remove the tubes from the magnet and resuspend the beads in **Elution Buffer** (Tris-HCl 10 mM, pH 8.0), low TE buffer, or TE buffer. **Note:** Resuspending the beads in less than 10 μ l may reduce the yield.
- 15) Load the plate on the magnet, incubate for 1 min, and transfer the supernatant (containing sample) to a new plate or tubes without disturbing the beads.

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Quality Control

Magnetic beads components passed stringent functional quality test.

Product Use Limitation


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