# GSure® Urine DNA Mini Kit

**#G46272A 50 preparations**

Store at Room Temperature

## Procedure:

1. Take a minimum of 5ml urine sample and centrifuge at 6000 x g for 10 mins at room temperature.
2. Discard the sup and add 250µl Buffer GDUR1. Incubate the tube at 70$^0$C for 15 min and vortex after every 2 min.
3. Add 250µl Buffer GDUR2 and mix by inverting the tube 4–6 times. Place the tube at 70$^0$C again for another 15 minutes.
4. Add 350µl Buffer GDUR3 and invert the tube immediately. Shake vigorously to mix the solutions, **DO NOT VORTEX AT THIS STAGE**. Vortex may cause shearing of genomic DNA.
5. Centrifuge for 10 minutes at 13,000 rpm (~8500xg) in a table-top microcentrifuge. A compact pellet will form.
6. Apply the supernatant to the GMini Spin Column by decanting or pipetting. ***Avoid mixing of cell debris with the supernatant*** *as* this may clog GMini spin Column thus lowering the DNA yield.
7. Centrifuge at 13,000 rpm (~8500xg) for 30–60 s. Discard the flow-through.
8. Wash GMini Spin Column by adding 600µl Membrane Wash Buffer* and centrifuging for 30–60 s as previously.
9. Discard the flow-through.
10. Repeat washing step.
11. Discard the flow-through, and centrifuge for an additional 2 minutes to remove residual wash buffer from membrane.

    ***This step is extremely important to ensure complete removal of ethanol. Presence of ethanol in purified DNA may inhibit subsequent enzymatic reactions.***

12. Place the GMini Spin Column in a clean 1.5 ml microcentrifuge tube (not provided). To elute DNA, add 50µl nuclease-free Water (provided) to the center of each GMini Spin Column, let stand for 1 minute, and centrifuge for 1 minute at maximum speed (~8500Xg) on a table top microcentrifuge.
13. Discard the column and collect the eluted DNA present in microcentrifuge tube.

- If required, increase the volume of GDUR1, GDUR2 and GDUR3 accordingly.
- **If any sediment found in any of the isolation buffers, warm the containers at 50°C until it dissolves.**

## Kit Contents:

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| GDUR1 Buffer | : 15ml |
| GDUR2 Buffer | : 15ml |
| GDUR3 Buffer | : 20ml |
| Membrane Wash Buffer | : 30ml |
| Spin Column | : 50pcs. |
| Nuclease-free Water | : 3ml |

## *Reconstitution of Membrane Wash Buffer:

Before using the kit for first time, add 45ml of absolute ethanol (molecular biology grade) with the provided wash buffer. Mix thoroughly by shaking. Once ethanol added, tighten the cap properly after each use.