ZYMO RESEARCH

*The Beauty of Science is to Make Things Simple*

# INSTRUCTION MANUAL

# *Quick*-DNA™ Urine Kit

Catalog No. **D3061**

## Highlights

- Purify cellular and/or cell-free DNA easily and reliably from up to 40 ml of urine.
- Uniquely formulated urine conditioning reagent allows stabilization of DNA in urine for up to 1 month at ambient temperature.
- Clean-Spin™ column technology ensures DNA is ready for all sensitive downstream applications including qPCR, DNA sequencing, arrays, and DNA methylation analysis.

## Contents






**ZYMO RESEARCH CORP.**

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Satisfaction of all Zymo Research products is guaranteed. If you are not satisfied with this product please call 1-888-882-9682.

## Product Contents

| ***Quick*-DNA™ Urine Kit** (Kit Size) | **D3061** (50 Preps.) | **Storage Temperature** |
|---|---|---|
| **Urine Conditioning Buffer** | 140 ml | Room Temp. |
| **Clearing Beads** | 1 ml | Room Temp. |
| **Urine Pellet Digestion Buffer** | 20 ml | Room Temp. |
| **Proteinase K*** | 2 x 20 mg | -20˚C (after mixing) |
| **Genomic Lysis Buffer**** | 50 ml | Room Temp. |
| **Urine DNA Prep Buffer** | 10 ml | Room Temp. |
| **Urine DNA Wash Buffer***** (concentrate) | 12 ml | Room Temp. |
| **DNA Elution Buffer** | 4 ml | Room Temp. |
| **Zymo-Spin™ IC-S Columns** | 50 | Room Temp. |
| **Collection Tubes** | 100 | Room Temp. |
| **Instruction Manual** | 1 | |

Note **-** Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability.

* Add 1,040 µl of **Proteinase K Storage Buffer** to reconstitute the lyophilized 20 mg **Proteinase K** at 20 mg/ml prior to use and store at -20˚C after use.

** Add beta-mercaptoethanol to a final dilution of 0.5% (v/v) i.e., 250 µl per 50 ml.

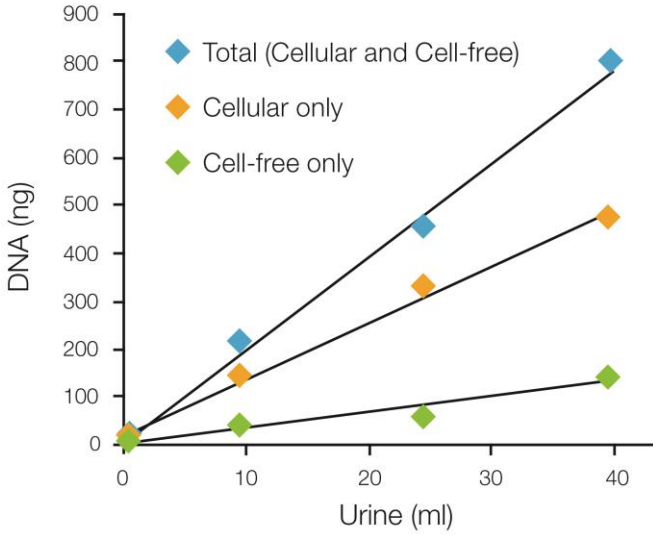
*** Add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **Urine DNA Wash Buffer** (concentrate).

## Product Specifications

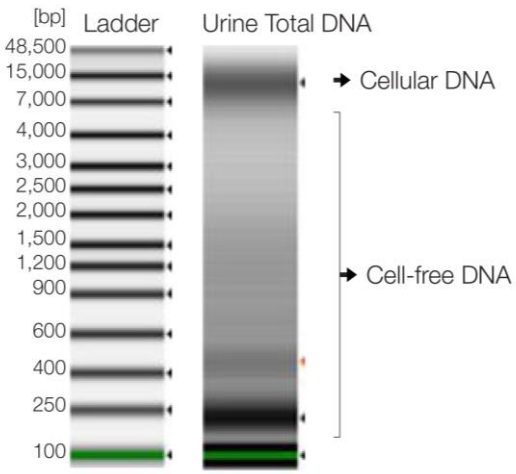
- **Sample Source** – Urine.
- **Sample Volume** – Up to 40 ml of urine per standard preparation. The sample volume can be scaled up or down if required.
- **DNA Quality**– High quality DNA can be used for downstream application, such as PCR, bisulfite treatment/methylation detection, array, etc.
- **DNA Yield** – The DNA binding capacity of the column is 5 µg. Note that DNA yield may vary depending on the urine itself. Female urine typically yields more DNA than male urine. Urine DNA from healthy female individual ranges on average from 6 - 1000 ng/ml. DNA from healthy male individual ranges on average from 2 - 20 ng/ml.
- **DNA Size** – Capable of recovering DNA fragments from 100 bp to 23 kb.
- **Equipment** – A centrifuge, a microcentrifuge, and a heat block / water bath.

## Product Description

The ***Quick*-DNA™ Urine Kit** is an innovative product designed for easy, reliable, and rapid isolation of cellular and/or cell-free DNA from up to 40 ml of urine. The product features a uniquely formulated urine DNA stabilization reagent that also functions as a precipitation reagent. After collection, total (cellular and cell-free) or cell-free urine can be stored at ambient temperature for up to one month by adding the **Urine Conditioning Buffer**. When ready to extract the urine DNA, just add the **Clearing Beads**, vortex, and centrifuge to collect the precipitate. Following precipitation, chemical lysis and enzymatic digestion are used to extract DNA from the precipitate. The DNA is subsequently purified and concentrated using **Zymo-Spin™ IC-S Columns**. Urine DNA isolated with the ***Quick*-DNA™ Urine Kit** is ideal for qPCR, array, methylation analysis[1], and other downstream applications.


**DNA yields increase linearly with increasing volumes of urine from healthy subjects extracted using the *Quick*-DNA™ Urine Kit**. DNA was isolated from 1 ml, 10 ml, 25 ml, and 40 ml urine. DNA concentration was quantified using the **Femto™ Human DNA Quantification Kit** (Zymo Research, E2005).




**Both cellular and cell-free DNA can be effectively purified from urine using the *Quick*-DNA™ Urine Kit**. 5 ml of urine from a healthy female donor was processed and DNA was eluted in 20 µl final volume. 1 µl of the sample was analyzed using a 2200 TapeStation.

**Notes:**

[1] Zymo Research offers the following for rapid and precise DNA methylation detection.

1.) **EZ DNA Methylation-Lightning™ Kit** (D5030, D5031, D5032, D5033, D5046, D5047)

2.) **EZ DNA Methylation-Direct™ Kit** (D5020, D5021, D5022, D5023, D5044, D5045)

3.) **EZ DNA Methylation-Gold™ Kit** (D5005, D5006, D5007, D5008, D5042, D5043)

4.) **5-mC DNA ELISA Kit** (D5325, D5326)

## Buffer Preparation

- ✓ Add beta-mercaptoethanol (user supplied) to the **Genomic Lysis Buffer** to a final dilution of 0.5% (v/v) i.e., 250 µl per 50 ml.
- ✓ Add 1,040 µl of **Proteinase K Storage Buffer** to reconstitute the lyophilized 20 mg **Proteinase K** at 20 mg/ml prior to use and store at -20˚C after use.
- ✓ Add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **Urine DNA Wash Buffer** (concentrate).

## Protocol

*The following protocol is designed for the isolation of DNA from up to 40 ml urine sample*[1]. *All steps should be performed at room temperature (20 - 30 ˚C).*

The protocol consists of three sections:

**(A)** Total (cellular and cell-free) DNA Precipitation,
**(B)** Protein Digestion, and
**(C)** DNA Purification.

### (A) Total (cellular and cell-free) DNA Precipitation

*(If only cellular or cell-free DNA is desired, refer to* ***Appendix A*** *on* ***page 6****)*

1. Transfer up to 40 ml urine[2] into a microcentrifuge tube or a conical tube.

   ***Note:*** *Use a microcentrifuge tube if processing ≤ 1 ml urine.*
   *Use a 15 ml conical tube if processing > 1 ml to14 ml urine.*
   *Use a 50 ml conical tube if processing > 14 ml to 40 ml urine.*

2. Add 70 µl **Urine Conditioning Buffer** for every 1 ml of urine (*e.g. Add 350 µl Urine Conditioning Buffer to 5 ml urine*). Mix the urine mixture well by vortexing.

   After adding and mixing urine with **Urine Conditioning Buffer**, urine can be stored up to 1 month at ambient temperature. At time of processing, mix the urine mixture well by vortexing and continue to the next step.

3. Add 10 µl **Clearing Beads**[3] if processing ≤ 14 ml urine and add 20 µl **Clearing Beads**[3] if processing >14 to 40 ml urine. Mix the urine mixture well by vortexing.

4. Centrifuge at 3,000 *x g* for 15 minutes.
   *(If isolating DNA from gram (+) bacteria, fungal, yeast, or other tough-to-lyse samples in urine, continue to* ***Appendix B*** *on* ***page 7****)*

**Notes:**

[1] The volume of urine sample can be scaled up or down if required.

[2] Warm up the urine at 37 ˚C for 5 - 10 minutes if the urine is visually cloudy. This may be caused by salt precipitation. If the urine does not turn clear, it may be caused by bacterial contamination.

[3] Mix **Clearing Beads** well before use by vortexing.

## (B) Protein Digestion

5. Without disturbing the pellet, slowly decant or pipette out the urine supernatant leaving behind 100 - 400 µl of pellet

   ***Recommended:*** *leave at least 200 µl of pellet if processing 15 - 40 ml urine.*

   ***Note:*** *you could adjust the volume to 100 - 400 µl by adding DNase/RNase-Free Water to the pellet.*

6. Add an equal volume of **Urine Pellet Digestion Buffer** to the pellet (*e.g. Add 100 µl Urine Pellet Digestion Buffer to 100 µl pellet*). Resuspend the pellet well by pipetting or vortexing.

7. Add 5% (v/v) of **Proteinase K** to the resuspended pellet (*e.g. Add 10 µl Proteinase K to 200 µl mixture*), mix well by vortexing, and incubate the pellet mixture at 55 ˚C for 30 minutes.

## (C) DNA Purification

*All centrifugation steps should be performed ≥ 16,000 x g, unless stated otherwise.*

8. Add 1 volume of **Genomic Lysis Buffer** to the digestion mixture (*e.g. Add 210 µl Genomic Lysis Buffer to 210 µl digestion mixture*) and mix well by vortexing.

9. Transfer the sample into a **Zymo-Spin™ IC-S Column** in a **Collection Tube**. Centrifuge for 1 minute. Discard flow-through. Repeat with the remaining mixture[1].

   ***Note:*** *The column may need to be loaded multiple times. Transfer 800 µl maximum for each load of mixture.*

10. Transfer the **Zymo-Spin™ IC-S Column** to a new **Collection Tube**.

11. Add 200 µl of **Urine DNA Prep Buffer** to the spin column. Centrifuge for 1 minute. Discard flow-through.

12. Add 700 µl **Urine DNA Wash Buffer** to the column and centrifuge for 1 minute. Discard flow-through. Repeat this step with 200 µl **Urine DNA Wash Buffer.**

13. Transfer the spin column to a DNase/RNase-free microcentrifuge tube. Add ≥ 10 µl[2] **DNA Elution Buffer**[3] or water directly on the column matrix and let stand for 3 - 5 minutes at room temperature. Centrifuge at full speed for 1 minute. The eluted DNA can be used immediately or stored at ≤ -20 ˚C for future use.

    ***Note:*** *If the concentration of the DNA is > 100 ng/µl in the first elution. Most of the DNA will be recovered on the first elution however the loading eluate from the first elution back onto the column, incubating for 3 - 5 minutes at room temperature, and centrifuging again can increase total yield*

**Notes:**

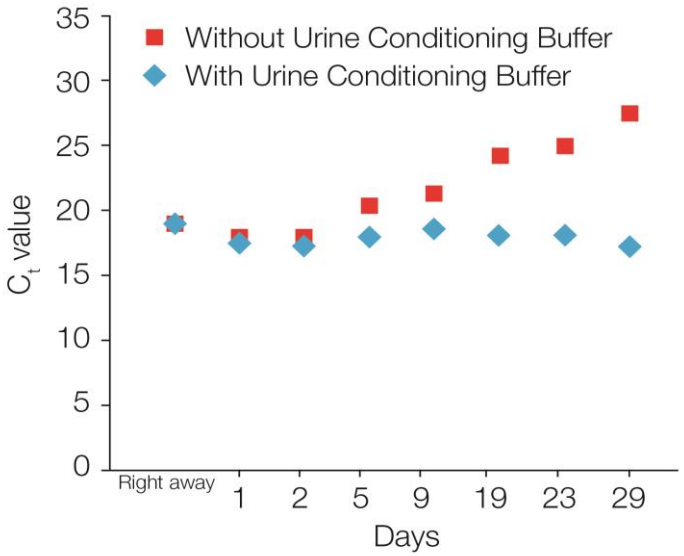
[1] **Clearing Beads** will be retained in the column.

[2] DNA yield may be increased by warming the **DNA Elution Buffer** to 65 ˚C before eluting and by performing a re-elution. Do not worry about the Clearing Beads that are retained at the ridge of the column. The DNA binding to the Clearing Beads are negligible. It is important to load the DNA Elution Buffer directly on the column matrix.

[3] **DNA Elution Buffer**: 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If water is used, make sure the pH is > 6.0.

## Protocol at a Glance

| | | | | |
|---|---|---|---|---|
| **DNA Precipitation** | Urine volume | ≤ 1 ml | > 1 - 14 ml | > 14 - 40 ml |
| | Tube to be used | microcentrifuge | 15 ml conical | 50 ml conical |
| | **Urine Conditioning Buffer** to be added | 70 µl / ml urine | | |
| | **Clearing Beads** to be added | 10 µl | 10 µl | 20 µl |
| | | Centrifuge at 3000 *x g* for 15 minutes | | |
| **Protein Digestion** ***(minimum pellet volume is listed)*** | Pellet volume to be left | 100 µl | 100 µl | 200 µl |
| | **Urine Pellet Digestion Buffer** to be added | 100 µl | 100 µl | 200 µl |
| | Reconstituted **Proteinase K** to be added | 10 µl | 10 µl | 20 µl |
| | | Incubate at 55 ˚C for 30 minutes | | |
| **DNA Purification** | **BIND: Genomic Lysis Buffer** to be added | 210 µl | 210 µl | 420 µl |
| | | Load to Column IC-S | | |
| | **WASH 1: Urine DNA Prep Buffer** to wash | 200 µl | | |
| | **WASH 2: Urine DNA Wash Buffer** to wash | 700 µl, 200 µl | | |
| | **ELUTE: DNA Elution Buffer** to be added | ≥ 10 µl | | |




**DNA in urine can be preserved for up to 1 month at ambient temperature.** Total (cellular and cell-free) urine DNA was incubated with and without **Urine Conditioning Buffer** for 29 days. For each sample, 5 ml urine from a healthy female donor was processed using the ***Quick*-DNA™ Urine Kit** and the $C_t$ values were determined by qPCR using the **Femto™ Human DNA Quantification Kit** (Zymo Research, E2005).

# Appendix A

## Isolating Cellular or Cell-free DNA Only

### 1. Cellular DNA only

i. Centrifuge up to 40 ml urine in a microcentrifuge tube or a conical tube at 3,000 *x g* for 15 minutes.

ii. Proceed to **Protocol** section **(B) Protein Digestion** on **page 4**,

***OR***

Proceed to **Appendix B** on **page 7** if isolating DNA from gram (+) bacteria, fungal, yeast, or other tough-to-lyse samples in urine.

### 2. Cell-free DNA only

i. Centrifuge up to 40 ml urine in a microcentrifuge tube or a conical tube at 3,000 *x g* for 15 minutes.

ii. Without disturbing the pellet, carefully transfer urine supernatant to a new microcentrifuge tube or a conical tube.

***Note**: Use a microcentrifuge tube if processing ≤ 1 ml cell-free urine.*
*Use a 15 ml conical tube if processing > 1 ml to14 ml cell-free urine.*
*Use a 50 ml conical tube if processing > 14 ml to 40 ml cell-free urine.*

iii. Add 70 µl **Urine Conditioning Buffer** for every 1 ml of urine supernatant (*e.g. Add 350 µl Urine Conditioning Buffer to 5 ml urine*) and mix well by vortexing.

After adding and mixing urine with **Urine Conditioning Buffer**, urine can be stored up to 1 month at ambient temperature. At time of processing, mix the urine mixture well by vortexing and continue to the next step.

iv. Add 10 µl **Clearing Beads**[1] if processing ≤ 14 ml urine and add 20 µl **Clearing Beads**[1] if processing >14 to 40 ml urine. Mix the urine mixture well by vortexing.

v. Centrifuge at 3000 *x g* for 15 minutes.

vi. Proceed to **Protocol** section **(B) Protein Digestion** on **page 4**,

**Notes:**

[1] Mix **Clearing Beads** well before use by vortexing.

**Notes:**

**[1]The following additional components are sold separately:**

**ZR BashingBead Lysis Tube (0.1 & 0.5 mm) -**
50 pack: S6012-50

**BashingBead Buffer -**
40 ml: D6001-3-40
150 ml: D6001-3-150

**Zymo-Spin III-F Filters -**
50 pack: C1057-50

**Collection Tubes -**
50 pack: C1001-50
500 pack: C1001-500

[2] DNA yield may be increased by warming the **DNA Elution Buffer** to 65 ˚C before eluting and by performing a re-elution.

# Appendix B

## Tough-to-Lyse Samples

Order the **ZR BashingBead Lysis Tubes, BashingBead Buffer, Zymo-Spin III-F Filters,** and additional **Collection Tubes** before proceeding[1]. The following protocol is an adapted protocol for lysing tough-to-lyse organisms in urine, such as gram (+) bacteria, yeast, and other fungi.

## Sample Lysis

1. Without disturbing the pellet, slowly decant or pipette out the urine supernatant leaving behind 100 - 400 µl of pellet. Transfer the pellet to a **ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)** and then add **BashingBead Buffer** to a final volume of 800 µl (*e.g. Add 700 µl of BashingBead Buffer to a 100 µl pellet).*

2. Secure in a bead beater fitted with a 2 ml tube holder assembly (e.g., Disruptor Genie™) and process at maximum speed for 5 minutes.

   ***Note:*** *Processing times may be as little as 40 seconds when using high-speed cell disrupters (e.g. the portable Xpedition™ Sample Processor, FastPrep®-24 or similar). See manufacturer's literature for operating information.*

3. Centrifuge the ZR BashingBead Lysis Tube at 10,000 *x g* for 1 minute.

4. Transfer 400 µl of the supernatant to a **Zymo-Spin™ III-F Filter** in a **Collection Tube**. Centrifuge at 10,000 *x g* for 1 minute. **Keep** flow-through and discard the filter.

## DNA Purification

*All centrifugation steps should be performed ≥ 16,000 x g, unless stated otherwise.*

5. Add 1,200 µl **Genomic Lysis Buffer** to the flow-through. Mix well by pipetting up and down several times. Transfer 800 µl of the mixture to the **Zymo-Spin™ IC-S Column** in a clean **Collection Tube**. Centrifuge for 1 minute. Discard the flow-through. Repeat with the remaining mixture.

6. Transfer the **Zymo-Spin™ IC-S Column** to a new **Collection Tube**.

   ***Note:*** *The column may need to be loaded multiple times. Transfer 800 µl maximum for each load of mixture.*

7. Add 200 µl **Urine DNA Prep Buffer** to the column. Centrifuge for 1 minute. Discard the flow-through.

8. Add 700 µl **Urine DNA Wash Buffer** to the column. Centrifuge for 1 minute. Discard the flow-through. Repeat this step with 200 µl **Urine DNA Wash Buffer**.

9. Transfer the column to a DNase/RNase-free microcentrifuge tube. Add ≥ 10 µl **DNA Elution Buffer**[2] directly to the column matrix and let stand for 3 - 5 minutes at room temperature. Centrifuge at full speed for 1 minute. The eluted DNA can be used immediately or stored at ≤ -20 ˚C for future use.

   ***Note:*** *If the concentration of the DNA is > 100 ng/µl in the first elution. Most of the DNA will be recovered on the first elution however the loading eluate from the first elution back onto the column, incubating for 3 - 5 minutes at room temperature, and centrifuging again can increase total yield*

# *Quick*-DNA™ Urine Kit

Catalog Nos. D3061

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## Quick Protocol

The quick protocol is designed for the isolation of total DNA from urine (both cellular and cell-free DNA).

See the Instruction Manual page 6 for selective isolation of cellular or cell-free DNA from urine.

See the Instruction Manual page 7 if processing tough-to-lyse samples such as yeast and bacteria from urine.

**Buffer Preparation:**

- ✓ Add beta-mercaptoethanol (user supplied) to the **Genomic Lysis Buffer** to a final dilution of 0.5% (v/v) i.e., 250 µl per 50 ml.
- ✓ Add 1,040 µl of **Proteinase K Storage Buffer** to reconstitute the lyophilized 20 mg **Proteinase K** at 20 mg/ml prior to use and store at -20˚C after use.
- ✓ Add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **Urine DNA Wash Buffer** (concentrate).

The following procedure should be performed at room temperature (15 - 30°C).

1. Transfer up to 40 ml urine into a microcentrifuge tube or a conical tube.

   ***Note**: Use a microcentrifuge tube if processing ≤ 1 ml urine.*
   *Use a 15 ml conical tube if processing > 1 ml to14 ml urine.*
   *Use a 50 ml conical tube if processing > 14 ml to 40 ml urine.*

2. Add 70 µl **Urine Conditioning Buffer** for every 1 ml of urine *(e.g. Add 350 µl Urine Conditioning Buffer to 5 ml urine).* Mix the urine mixture well by vortexing.

   After adding and mixing urine with **Urine Conditioning Buffer**, urine can be stored up to 1 month at ambient temperature. At time of processing, mix the urine mixture well by vortexing and continue to step 3.

3. Add 10 µl **Clearing Beads** if processing ≤ 14 ml urine and add 20 µl **Clearing Beads** if processing >14 to 40 ml urine. Mix the urine mixture well by vortexing.

4. Centrifuge at 3,000 *x g* for 15 minutes.

# *Quick*-DNA™ Urine Kit

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5. Without disturbing the pellet, slowly decant or pipette out the urine supernatant leaving behind 100 - 400 µl of pellet.

   ***Recommended:*** *leave at least 200 µl of pellet if processing 15 - 40 ml urine.*

   ***Note:*** *you could adjust the volume to 100 - 400 µl by adding DNase/Rnase-Free Water to the pellet.*

6. Add an equal volume of **Urine Pellet Digestion Buffer** to the pellet *(e.g. Add 100 µl Urine Pellet Digestion Buffer to 100 µl pellet).* Resuspend the pellet well by pipetting or vortexing.

7. Add 5% (v/v) of **Proteinase K** to the resuspended pellet *(e.g. Add 10 µl Proteinase K to 200 µl mixture),* mix well by vortexing, and incubate the pellet mixture at 55 ˚C for 30 minutes.

8. Add 1 volume of **Genomic Lysis Buffer** to the digestion mixture *(e.g. Add 210 µl Genomic Lysis Buffer to 210 µl digestion mixture)* and mix well by vortexing.

9. Transfer the sample into a **Zymo-Spin™ IC-S Column** in a **Collection Tube**. Centrifuge for 1 minute. Discard flow-through. Repeat with the remaining mixture.

   ***Note:*** *The column may need to be loaded multiple times. Transfer 800 µl maximum for each load of mixture.*

10. Transfer the **Zymo-Spin™ IC-S Column** to a new **Collection Tube**.

11. Add 200 µl of **Urine DNA Prep Buffer** to the spin column. Centrifuge for 1 minute. Discard flow-through.

12. Add 700 µl **Urine DNA Wash Buffer** to the column and centrifuge for 1 minute. Discard flow-through. Repeat this step with 200 µl **Urine DNA Wash Buffer**.

13. Transfer the spin column to a DNase/RNase-free microcentrifuge tube. Add ≥ 10 µl **DNA Elution Buffer** or water directly on the column matrix and let stand for 3 - 5 minutes at room temperature. Centrifuge at full speed for 1 minute.



**For the full Instruction Manual, visit**
**http://www.zymoresearch.com/m/D3061**

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## Troubleshooting

*For **Technical Assistance**, please contact 1-888-882-9682 or E-mail tech@zymoresearch.com.*

| Problem | Possible Causes and Suggested Solution |
|---|---|
| **Low DNA Yield** | *Volume Input*<br>• For low DNA containing urine, you can increase the input volume to as high as 40 ml.<br><br>*Increasing DNA Yields*<br>• The total yield may be improved by eluting the DNA with DNA Elution Buffer pre-heated to 60 - 70°C.<br>• Loading the eluate from the first elution back onto the column, incubate for 3 - 5 minutes at room temperature, and centrifuge at full speed can maximize recovery.<br><br>*Incomplete Lysis/Digestion*<br>• Ensure Proteinase K digestions are performed at 55 ˚C as indicated. It is possible to extend digestion times to 1 hour if samples are high in protein.<br>• Vortex samples longer after the addition of Genomic Lysis Buffer to ensure that the lysate is homogenous.<br><br>*Variety in Urine Samples*<br>• Urine DNA from healthy female individual ranges on average from 6 - 1000 ng/ml.<br>• DNA from healthy male individual ranges on average from 2 - 20 ng/ml. |
| **Urine Storage** | *Short Term Storage*<br>• Urine samples could be stored up to 1 month at ambient temperature by adding Urine Conditioning Buffer. Add 70 µl Urine Conditioning Buffer for every 1 ml of urine and mix thoroughly by vortexing. At time of processing, mix the urine well by vortexing and continue by adding the Clearing Beads.<br><br>*Long Term Storage*<br>• If you want to store the urine samples for more than 1 month, we recommend pelleting after adding the Urine Conditioning Buffer. Add 70 µl Urine Conditioning Buffer for every 1 ml of urine and mix thoroughly by vortexing. Centrifuge at 3,000 *x g* for 15 minutes. Discard urine supernatant and store the pellet at -20 ˚C or -80 ˚C.<br><br>Keep in mind that after adding Urine Conditioning Buffer, the cellular and cell-free fractions of the urine DNA cannot be separated. If required, separate the cellular and cell-free urine DNA fractions of the urine by following **Appendix A page 6**. |

**Sample Condition**

*Cloudy urine*

- This may be caused by salt precipitation. Warm up the urine at 37 ˚C for 5 - 10 minutes if the urine is visually cloudy. If the urine does not turn clear, it may be caused by bacterial contamination.

*Clearing Beads in the flow through or eluate*

- Urine with high amount of cellular content may cause clearing beads to pass through the spin column.

- If the Clearing Beads are present in the eluate, centrifuge the eluate at full speed for 1 minute and transfer the supernatant to a DNase/RNase-free microcentrifuge tube for storage or analysis.

*The presence of oil-like solution after the 15 minutes 3,000 x g centrifugation*

- We recommend removing the oil-like solution before adding the Urine Pellet Digestion Buffer and proceed with Protein Digestion Section on page 4.

## Ordering Information

| Product Description | Catalog No. | Kit Size |
|---|---|---|
| **Quick-DNA™ Urine Kit** | D3061 | 50 Preps. |

| For Individual Sale | Catalog No. | Amount |
|---|---|---|
| **Proteinase K** | D3001-2-5<br>D3001-2-20 | 5 mg<br>20 mg |
| **Genomic Lysis Buffer** | D3004-1-50<br>D3004-1-100 | 50 ml<br>100 ml |
| **DNA Elution Buffer** | D3004-4-1<br>D3004-4-4<br>D3004-4-10<br>D3004-4-50 | 1 ml<br>4 ml<br>10 ml<br>50 ml |
| **Urine Conditioning Buffer** | D3061-1-140 | 140 ml |
| **Clearing Beads** | D3061-2-1 | 1 ml |
| **Urine Pellet Digestion Buffer** | D3061-3-20 | 20 ml |
| **Urine DNA Prep Buffer** | D3061-4-10 | 10 ml |
| **Urine DNA Wash Buffer** (concentrate) | D3061-5-12 | 12 ml |
| **Zymo-Spin™ IC-S Columns** | C1015-50 | 50 columns |
| **Collection Tubes** | C1001-50<br>C1001-500<br>C1001-1000 | 50 tubes<br>500 tubes<br>1,000 tubes |

| **Supplementary Components**<br>(for tough-to-lyse samples) | Catalog No. | Amount |
|---|---|---|
| **ZR BashingBead Lysis Tube (0.1 & 0.5 mm)** | S6012-50 | 50 tubes |
| **BashingBead Buffer** | D6001-3-40<br>D6001-3-150 | 40 ml<br>150 ml |
| **Zymo-Spin III-F Filters** | C1057-57 | 50 pack |
| **Collection Tubes** | C1001-50<br>C1001-500 | 50 tubes<br>500 tubes |

THE Epigenetics COMPANY™

# *Related Products from Zymo Research*

| Product | Description | Kit Size (Preps) | Catalog No. (Format) |
|---|---|---|---|
| | ***DNA Methylation Detection*** | | |
| **EZ DNA Methylation-Lightning™ Kit** | Complete bisulfite conversion in about an hour using a unique liquid format conversion reagent that requires no preparation. Fast-Spin technology ensures ultra-pure, converted DNA for subsequent DNA methylation analysis. Magnetic bead format for adaptation to automated liquid handling platforms. | 50 Rxns.<br>200 Rxns.<br>2x96 Rxns.<br>2x96 Rxns.<br>4x96 Rxns.<br>8x96 Rxns. | **D5030** (spin column)<br>**D5031** (spin column)<br>**D5032** (shallow-well plate)<br>**D5033** (deep-well plate)<br>**D5046** (magnetic bead)<br>**D5047** (magnetic bead) |
| **EZ DNA Methylation-Direct™ Kit** | Features simple and reliable DNA bisulfite conversion directly from blood, tissue (FFPE/LCM), and cells without the prerequisite for DNA purification in as little as 4-6 hrs. The increased sensitivity of this kit makes it possible to amplify bisulfite converted DNA from as few as 10 cells or 50 pg DNA. Magnetic bead format for adaptation to automated liquid handling platforms. | 50 Rxns.<br>200 Rxns.<br>2x96 Rxns.<br>2x96 Rxns.<br>4x96 Rxns.<br>8x96 Rxns. | **D5020** (spin column)<br>**D5021** (spin column)<br>**D5022** (shallow-well plate)<br>**D5023** (deep-well plate)<br>**D5044** (magnetic bead)<br>**D5045** (magnetic bead) |
| **EZ DNA Methylation-Gold™ Kit** | For the fast (3 hr.) conversion of unmethylated cytosines in DNA to uracil via heat/chemical-denaturation of DNA and a specially designed CT Conversion Reagent. Fast-Spin technology ensures ultra-pure, converted DNA for subsequent DNA methylation analysis. Magnetic bead format for adaptation to automated liquid handling platforms. | 50 Rxns.<br>200 Rxns.<br>2x96 Rxns.<br>2x96 Rxns.<br>4x96 Rxns.<br>8x96 Rxns | **D5005** (spin column)<br>**D5006** (spin column)<br>**D5007** (shallow-well plate)<br>**D5008** (deep-well plate)<br>**D5042** (magnetic bead)<br>**D5043** (magnetic bead) |
| **5-mC DNA ELISA Kit** | A convenient and powerful tool that allows the researcher to accurately quantitate 5-mC in any DNA sample in less than 3 hours. The kit features a unique anti-5-Methylcytosine monoclonal antibody that is both sensitive and specific for 5-mC. | 1x96 Rxns.<br>2x96 Rxns. | **D5325**<br>**D5326** |
| **Methylated-DNA IP Kit** | IP with a highly specific anti-5-methylcytosine monoclonal antibody. Designed for the enrichment of 5-methylcytosine-containing DNA from any pool of fragmented genomic DNA for use in genome-wide methylation analysis. | 10 Rxns. | **D5101** |
| | ***Other…*** | | |
| **Universal Methylated Human DNA Standards** | Human (male) genomic DNA having all CpG sites methylated. To be used for the evaluation of bisulfite-mediated conversion of DNA. Supplied with a control primer set. | 1 set | **D5011** |
| **Zymo*Taq*™ DNA Polymerase** | ZymoTaq™ "hot start" DNA Polymerase is specifically designed for the amplification of "difficult" DNA templates including: bisulfite-treated DNA for methylation detection. The product generates specific amplicons with little or no by-product formation. Available either as a single buffer premix or as a polymerase system with components provided separately. | 50 Rxns.<br>200 Rxns.<br>50 Rxns.<br>200 Rxns. | **E2001** (system)<br>**E2002** (system)<br>**E2003** (premix)<br>**E2004** (premix) |
| **Femto™ Human DNA Quantification Kit** | Highly specific detection and quantification of human DNA with increased accuracy, precision, and sensitivity. | 100 Rxns. | **E2005** |
| **Femto™ Bacterial DNA Quantification Kit** | Highly specific detection and quantification of bacterial DNA with increased accuracy, precision, and sensitivity. | 100 Rxns. | **E2006** |
| **Femto™ Fungal DNA Quantification Kit** | Highly specific detection and quantification of fungal DNA with increased accuracy, precision, and sensitivity. | 100 Rxns. | **E2007** |

**Please visit our website to see our complete line-up of products.**

# DNA PURIFICATION

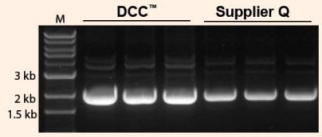

**What is Clean-Spin™ Technology?**
The spin columns from Zymo Research have been designed to ensure complete elution with no binding/wash buffer carryover. The result is ultra-pure inhibitor-free DNA and RNA.

## Purify DNA from PCR & other sources

### DNA Clean & Concentrator™ (DCC™)

- ✓ Recovery of ultra-pure DNA that is free of salts and contaminants.
- ✓ Small (≥6 µl) elution volume.
- ✓ DNA is ideal for ligation, PCR, Next-Gen sequencing, etc.

| Product | Size (Cat. No.) |
|---|---|
| DNA Clean & Concentrator™-5 | 50 Preps. (D4013)<br>200 Preps. (D4014) |
| ZR-96 DNA Clean & Concentrator™-5 | 2 x 96 Preps. (D4023)<br>4 x 96 Preps. (D4024) |
| Genomic DNA Clean & Concentrator™ | 25 Preps. (D4010)<br>100 Preps. (D4011) |



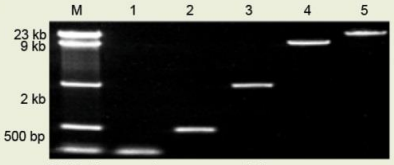

High efficiency DNA recovery with the DCC™-5 compared to Supplier Q .

## Boost DNA recoveries from agarose gels to >80%

### Zymoclean™ Gel DNA Recovery

- ✓ Rapid (15 min.) recovery of ultra-pure DNA from agarose gels in ≥6 µl.
- ✓ Ultra-pure DNA ideal for DNA ligation, sequencing, etc.
- ✓ Format also available for large DNA >20 kb.

| Product | Size (Cat. No.) |
|---|---|
| Zymoclean™ Gel DNA Recovery Kit | 50 Preps. (D4001)<br>200 Preps. (D4002) |
| Zymoclean™ Large Fragment DNA Recovery Kit | 25 Preps. (D4045)<br>100 Preps. (D4046) |




DNA fragments recovered from an agarose gel using the Zymoclean™ Gel DNA Recovery Kit. Lanes: M: DNA Ladder; 1-5: individual ladder DNA fragments.

## Recover transfection-quality plasmid DNA directly from culture

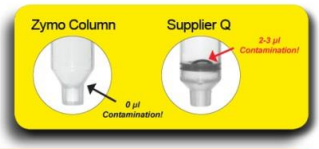
### Zyppy™ Plasmid Prep Kits

- ✓ The fastest, simplest method available for purifying high quality plasmid DNA from *E. coli.*
- ✓ Pellet-Free™ procedure omits conventional cell-pelleting and resuspension steps.
- ✓ Transfection quality plasmid DNA directly from culture in under 15 minutes.

**Easy, Pellet-free Procedure:** Add Lysis Buffer ***Directly*** to Bacterial Culture


| Product | Size (Cat. No.) |
|---|---|
| Zyppy™ Plasmid Miniprep Kit | 50 Preps. (D4036)<br>100 Preps. (D4019)<br>400 Preps. (D4020)<br>800 Preps. (D4037) |


# RNA PURIFICATION



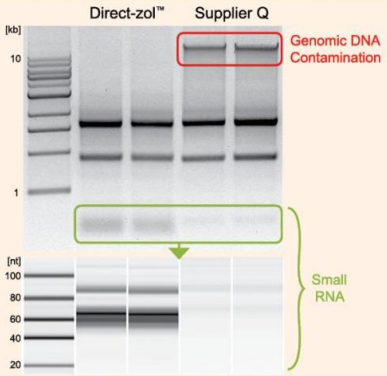

## Get RNA *directly* from TRIzol® without phase separation

### Direct-zol™ RNA

- ✓ For purification of high-quality small and large RNA *directly* from TRIzol®, TRI Reagent®, or similar.
- ✓ Bypasses phase separation and precipitation procedures allowing for unbiased recovery of miRNA

| Product | Size (Cat. No.) |
|---|---|
| Direct-zol™ RNA MiniPrep | 50 Preps. (R2050)<br>50 Preps. (R2051)*<br>200 Preps. (R2052)<br>200 Preps. (R2053)* |
| *96-well and MagBead formats also available!* | |

DNase I included in all kits.
* Supplied with *TRI-Reagent®*



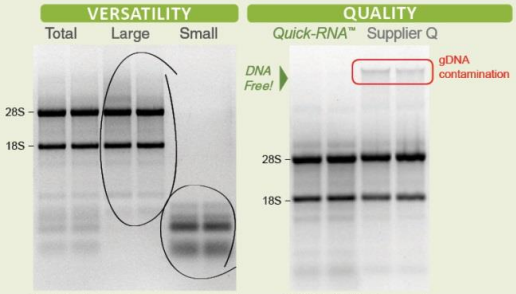

High-quality small and large RNA are effectively recovered with the Direct-zol™ kit. RNA is ***DNA-free***.

## Isolate DNA-free RNA from 1 to $10^7$ cells in minutes

### *Quick-RNA*™

- ✓ Isolation of total, large, or small RNA – *You decide!*
- ✓ Ultra clean, high-quality RNA from a single cell to $10^7$ cells.
- ✓ DNA-free RNA ideal for any downstream application – *DNase I included.*

| Product | Size (Cat. No.) |
|---|---|
| *Quick-RNA*™ MicroPrep | 50 Preps. (R1050)<br>200 Preps. (R1051) |
| *Quick-RNA*™ MiniPrep | 50 Preps. (R1054)<br>200 Preps. (R1055) |
| ZR-96 *Quick-RNA*™ | 2 x 96 Preps. (R1052)<br>4 x 96 Preps. (R1053) |




Isolate total, large, or small RNA with the *Quick-RNA*™ kit.

RNA is DNA-free using the *Quick-RNA*™ kit

## Purify RNA from enzymatic and labeling reactions in 5 minutes

### RNA Clean & Concentrator™

- ✓ Recover ultra-pure RNA in small (≥6 µl) elution volumes.
- ✓ Compatible with TRIzol®, phenol, choloform, and RNase inhibitors (RNAlater®).
- ✓ RNA is ideal for RT-PCR, q-PCR, hybridization, arrays, RNA interference, etc.

| Product | Size (Cat. No.) |
|---|---|
| RNA Clean & Concentrator™-5 | 50 Preps. (R1015)<br>200 Preps. (R1016) |
| RNA Clean & Concentrator™-25 | 50 Preps. (R1017)<br>100 Preps. (R1018) |
| ZR-96 RNA Clean & Concentrator™ | 2x96 well plates (R1080) |
| DNA-Free RNA Kit™ | 50 Preps. (R1013)<br>200 Preps. (R1014) |

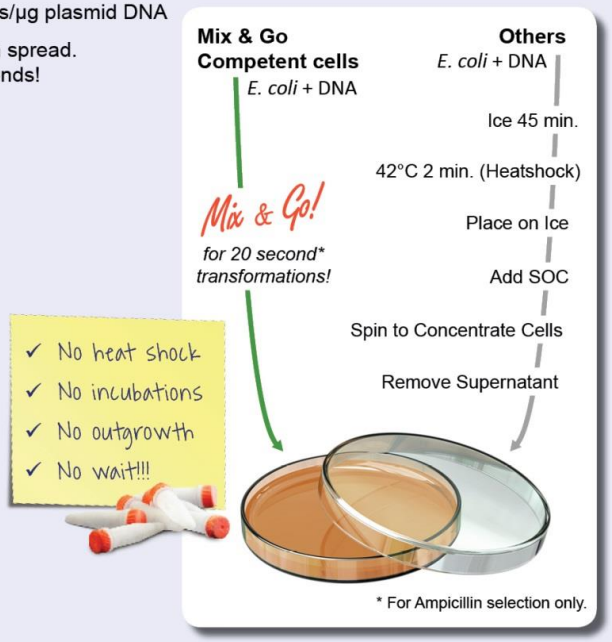
# OTHER INNOVATIVE PRODUCTS FROM ZYMO RESEARCH...

## Competent cells for transformations *without* heat shock!

### *Mix & Go!* Pre-made Competent *E. Coli*

- ✓ High efficiency: $10^8$-$10^9$ transformants/µg plasmid DNA
- ✓ Just *Mix & Go!* Simply add DNA then spread. Transformation in as little as 20 seconds!

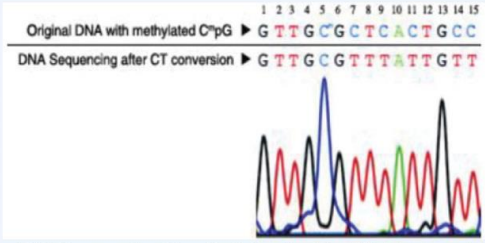
| Product | Size (Cat. No.) |
|---|---|
| Zymo 5α (Same as DH5α) | 10 x 100 µl aliquots (T3007)<br>96 x 50 µl aliquots (T3009)<br>96 x 50 µl aliquots PCR-plate (T3010) |
| Zymo 10B (Same as DH10B) | 10 x 100 µl aliquots (T3019)<br>96 x 50 µl aliquots (T3020) |
| JM109 | 10 x 100 µl aliquots (T3003)<br>96 x 50 µl aliquots (T3005) |
| HB101 | 10 x 100 µl aliquots (T3011)<br>96 x 50 µl aliquots (T3013) |
| C600 | 10 x 100 µl aliquots (T3015) |
| TG1 | 10 x 100 µl aliquots (T3017) |



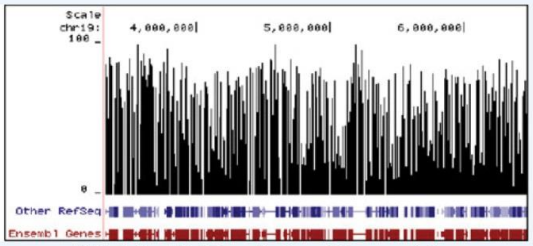

## The fastest method for complete bisulfite conversion of DNA

### *EZ DNA Methylation-Lightning™ Kits*

- ✓ The next generation of bisulfite conversion technology by the most cited provider in the industry
- ✓ Guarantees high conversion efficiencies of cytosine (>99.5%)
- ✓ Maintains the highest template integrity following bisulfite conversion
- ✓ Recovered DNA is ideal for PCR, MSP, array, bisulfite, and next-generation sequencing.




**DNA Sequencing Results Following Bisulfite Treatment**




**Methylation Plot From Reduced Representation Bisulfite Sequencing (RRBS)**

| Product | | Size (Cat. No.) |
|---|---|---|
| EZ DNA Methylation-Lightning™ Kit | | 50 rxns. (D5030)<br>200 rxns. (D5031) |
| EZ-96 DNA Methylation-Lightning™ Kit | Shallow-Well<br>Deep-Well | 2 x 96 rxns. (D5032)<br>2 x 96 rxns. (D5033) |
| EZ-96 DNA Methylation-Lightning™ MagPrep | | 4 x 96 rxns. (D5046)<br>8 x 96 rxns. (D5047) |