



ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

EZ DNA Methylation-Gold™ Kit

Catalog Nos. **D5005 & D5006**

Highlights

- Complete bisulfite conversion of GC-rich DNA in less than 3 hours.
- A coupled heat denaturation/conversion reaction step streamlines the conversion of unmethylated cytosines into uracil.
- DNA precipitations are omitted. Instead, DNA is cleaned and desulphonated in a single step using state-of-the-art spin columns.
- Eluted, ultra-pure DNA is ideal for use in subsequent molecular-based analyses.

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Product Contents:

EZ DNA Methylation-Gold™ Kit	D5005	D5006	Storage Temperature
CT Conversion Reagent*	50 rxns.	200 rxns.	Room Temp.
M-Dilution Buffer	1.5 ml	7 ml	Room Temp.
M-Dissolving Buffer	500 µl	1.2 ml	Room Temp.
M-Binding Buffer	30 ml	125 ml	Room Temp.
M-Wash Buffer**	6 ml	24 ml	Room Temp.
M-Desulphonation Buffer	10 ml	40 ml	Room Temp.
M-Elution Buffer	1 ml	4 ml	Room Temp.
Zymo-Spin™ IC Columns	50 ct.	200 ct.	Room Temp.
Collection Tubes	50 ct.	200 ct.	Room Temp.
Instruction Manual	1	1	-

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

* 900 µl water, 300 µl **M-Dilution Buffer**, and 50 µl **M-Dissolving Buffer** must be added per tube of **CT Conversion Reagent** prior to use.

** Add 24 ml of 100% ethanol to the 6 ml **M-Wash Buffer** concentrate (D5005) or 96 ml of 100% ethanol to the 24 ml **M-Wash Buffer** concentrate (D5006) before use.

EZ DNA Methylation-Gold™ Kit technologies are patent pending.

Use of Methylation Specific PCR (MSP) is protected by US Patents 5,786,146 & 6,017,704 & 6,200,756 & 6,265,171 and International Patent WO 97/46705. No license under these patents to use the MSP process is conveyed expressly or by implication to the purchaser by the purchase of this product.

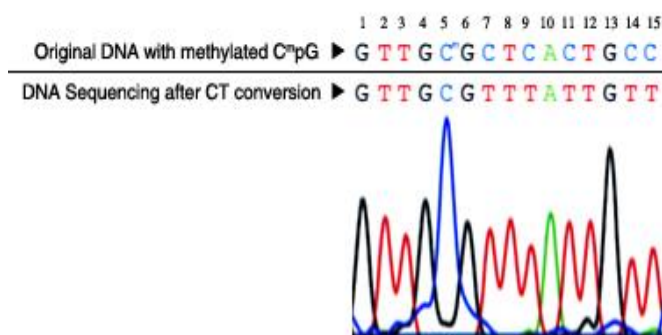
Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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Introduction to DNA Methylation:

DNA methylation is a naturally occurring event in both prokaryotic and eukaryotic organisms. In prokaryotes DNA methylation provides a way to protect host DNA from digestion by restriction endonucleases that are designed to eliminate foreign DNA, and in higher eukaryotes DNA methylation functions in the regulation/control of gene expression (1). It has been demonstrated that aberrant DNA methylation is a widespread phenomenon in cancer and may be among the earliest changes to occur during oncogenesis (2). DNA methylation has also been shown to play a central role in gene imprinting, embryonic development, X-chromosome gene silencing, and cell cycle regulation. In many plants and animals, DNA methylation consists of the addition of a methyl group to the fifth carbon position of the cytosine pyrimidine ring via a methyltransferase enzyme (3). The majority of DNA methylation in mammals occurs in 5'-CpG-3' dinucleotides, but other methylation patterns do exist. In fact, about 80 percent of all 5'-CpG-3' dinucleotides in mammalian genomes are found to be methylated, whereas the majority of the twenty percent that remain unmethylated are within promoters or in the first exons of genes.

The ability to detect and quantify DNA methylation efficiently and accurately has become essential for the study of cancer, gene expression, genetic diseases, as well as many other important aspects of biology. To date, a number of methods have been developed to detect/quantify DNA methylation including: high-performance capillary electrophoresis (4) and methylation-sensitive arbitrarily primed PCR (5). However, the most common technique used today remains the bisulfite conversion method (6). This technique involves treating methylated DNA with bisulfite, which converts unmethylated cytosines into uracil. Methylated cytosines remain unchanged during the treatment. Once converted, the methylation profile of the DNA can be determined by PCR amplification followed by DNA sequencing (see below).



DNA sequencing results following bisulfite treatment. DNA with methylated C^mpG at nucleotide position #5 was processed using the **EZ DNA Methylation™ Kit**. The recovered DNA was amplified by PCR and then sequenced directly. The methylated cytosine at position #5 remained intact while the unmethylated cytosines at positions #7, 9, 11, 14 and 15 were completely converted into uracil following bisulfite treatment and detected as thymine following PCR.

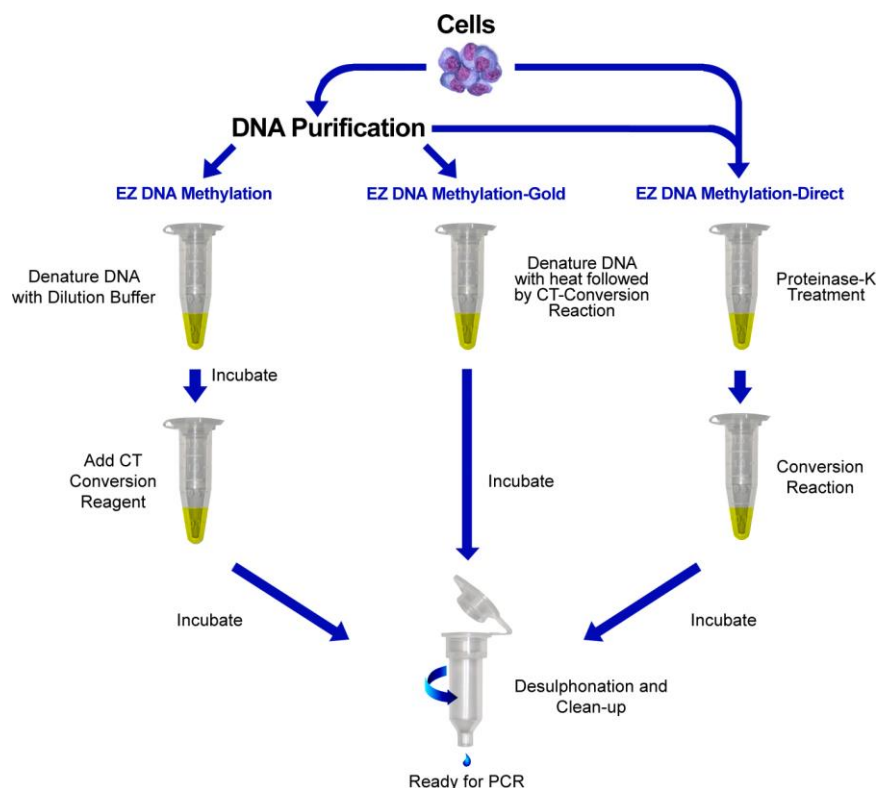
References:

1. Costello JF, Plass CJ. *Med. Genet.* 2001; 38(5): 285-303.
2. Stirzaker C. *Cancer Res.* 1997; 57(11): 2229-2237.
3. Adams RL. *Bioessays.* 1995; 17(2): 139-145.
4. Fraga MF, *et al.* *Electrophoresis.* 2000; 21(14): 2990-2994.
5. Gonzalgo ML. *Cancer Res.* 1997; 57(4): 594-599.
6. Frommer M. *Proc. Natl. Acad. Sci. USA.* 1992; 89(5): 1827-1831.

Product Description:

The **EZ DNA Methylation-Gold™ Kit** is a refinement of our popular **EZ DNA Methylation™ Kit**. The **EZ DNA Methylation-Gold™ Kit** integrates DNA denaturation and bisulfite conversion processes into one-step. This is accomplished using temperature denaturation to replace chemical denaturation with sodium hydroxide in the previous protocol. Also, the kit has been streamlined for high yield recovery of DNA following DNA bisulfite conversion. Both kits are based on a three-step reaction process between cytosine and sodium bisulfite resulting in cytosine being converted into uracil. The **EZ DNA Methylation-Gold™** and **EZ DNA Methylation™ Kits** share innovative in-column desulphonation technology that eliminates cumbersome DNA precipitation steps while providing researchers consistent results every time. The kits have been designed to minimize template degradation, loss of DNA during treatment and clean-up, and to provide complete conversion of unmethylated cytosines. Recovered DNA is ideal for PCR amplification for downstream analyses including endonuclease digestion, sequencing, microarrays, etc.

An outline comparing the **EZ DNA Methylation-Gold™ Kit** procedure to Zymo Research's other methylation kits is shown below.



Outline of the **EZ DNA Methylation™**, **EZ DNA Methylation-Gold™** and **EZ DNA Methylation-Direct™** Kit procedures.

Note: 96-Well spin-plate formats are available for processing larger numbers of samples. Also, MagPrep kits are available (p. 8) for adaptation to liquid handling robots (e.g., Tecan – Freedom EVO®) and automated sample prep.

Selected EZ DNA Methylation™ Kit Citations:

1. Ehrlich M, *et al.* Nuc. Acids Res. 2007; 35 (5): e29
2. Kaneda M, *et al.* Nature. 2004; 429: 900-903
3. Zhang F, *et al.* Proc. Natl. Acad. Sci. USA. 2007; 104 (11): 4395-4400.
4. Oda M, *et al.* Genes & Dev. 2006; 20: 3382-3394.
5. England RPM, *et al.* Nature Meth. 2005; 2: 1-2.

Specifications:

- **DNA Input:** Samples containing 500 pg - 2 µg of DNA. For optimal results, the amount of input DNA should be from 200 to 500 ng.
- **Conversion Efficiency:** > 99% of non-methylated C residues are converted to U; > 99% protection of methylated cytosines.
- **DNA Recovery:** > 75%

Reagent Preparation:

- **Preparation of CT Conversion Reagent**

The **CT Conversion Reagent** supplied within this kit is a solid mixture and must be prepared prior to first use. Prepare as follows:

1. Add 900 µl water, 300 µl of **M-Dilution Buffer**, and 50 µl **M-Dissolving Buffer** to a tube of **CT Conversion Reagent**.
2. Mix at room temperature with frequent vortexing or shaking for 10 minutes.

Note: It is normal to see trace amounts of undissolved reagent in the **CT Conversion Reagent**. Each tube of **CT Conversion Reagent** is designed for 10 separate DNA treatments.

Storage: The **CT Conversion Reagent** is light sensitive, so minimize its exposure to light. For best results, the **CT Conversion Reagent** should be used immediately following preparation. If not used immediately, the **CT Conversion Reagent** solution can be stored overnight at room temperature, one week at 4°C, or up to one month at -20°C. Stored **CT Conversion Reagent** solution must be warmed to 37°C, then vortexed prior to use.

- **Preparation of M-Wash Buffer**

Add 24 ml of 100% ethanol to the 6 ml **M-Wash Buffer** concentrate (D5005) or 96 ml of 100% ethanol to the 24 ml **M-Wash Buffer** concentrate (D5006) before use.

Protocol:

*For DNA volumes >20 µl, an adjustment needs to be made during the preparation of the **CT Conversion Reagent**. The amount of water is decreased 100 µl for each 10 µl increase in DNA sample volume. For example, for a 40 µl DNA sample, 700 µl of water is added to make the **CT Conversion Reagent**. The volume of **CT Conversion Reagent** added to the sample must also be decreased by the same volume as the sample is increased, total reaction volume remains 150 µl. The maximum DNA sample volume to be used for each conversion reaction is 50 µl. Do not adjust the volumes of either the **M-Dissolving Buffer** or **M-Dilution Buffer**.

The capacity of the collection tube with the column inserted is 800 µl. Empty the collection tube whenever necessary to prevent contamination of the column contents by the flow-through.

Alternatively, water or TE (pH ≥ 6.0) can be used for elution if required for your experiments.

1. Add 130 µl of the **CT Conversion Reagent** to 20 µl* of your DNA sample in a PCR tube. If the volume of the DNA sample is less than 20 µl, make up the difference with water. Mix the sample by flicking the tube or pipetting the sample up and down, then centrifuge the liquid to the bottom of the tube.
2. Place the sample tube in a thermal cycler and perform the following steps:
 1. 98°C for 10 minutes
 2. 64°C for 2.5 hours
 3. 4°C storage for up to 20 hours

Note: The 4°C storage step is *optional*. For some samples, alternative parameters may yield improved results (see Appendix). If you have been using this kit with good results using different reaction conditions than described above, you can continue using those same conditions.

3. Add 600 µl of **M-Binding Buffer** to a **Zymo-Spin™ IC Column** and place the column into a provided **Collection Tube**.
4. Load the sample (from Step 2) into the **Zymo-Spin™ IC Column** containing the **M-Binding Buffer**. Close the cap and mix by inverting the column several times.
5. Centrifuge at full speed ($\geq 10,000 \times g$) for 30 seconds. Discard the flow-through.
6. Add 100 µl of **M-Wash Buffer** to the column. Centrifuge at full speed for 30 seconds.
7. Add 200 µl of **M-Desulphonation Buffer** to the column and let stand at room temperature (20-30°C) for 15-20 minutes. After the incubation, centrifuge at full speed for 30 seconds.
8. Add 200 µl of **M-Wash Buffer** to the column. Centrifuge at full speed for 30 seconds. Add another 200 µl of **M-Wash Buffer** and centrifuge for an additional 30 seconds.
9. Place the column into a 1.5 ml microcentrifuge tube. Add 10 µl of **M-Elution Buffer** directly to the column matrix. Centrifuge for 30 seconds at full speed to elute the DNA.

The DNA is ready for immediate analysis or can be stored at or below -20°C for later use. For long term storage, store at or below -70°C. We recommend using 1-4 µl of eluted DNA for each PCR, however, up to 10 µl can be used if necessary. The elution volume can be > 10 µl depending on the requirements of your experiments, but small elution volumes will yield more concentrated DNA.

Frequently Asked Questions:

Q: Should the input DNA be dissolved in TE, water, or some other buffer prior to its conversion?

A: *Water, TE or modified TE buffers can be used to dissolve the DNA and do not interfere with the conversion process.*

Q: Which Taq polymerase(s) do you recommend for PCR amplification of converted DNA?

A: *We recommend a “hot start” DNA polymerase (e.g., ZymoTaq™, page 9).*

Q: Why are there two different catalog numbers for the EZ-96 DNA Methylation-Gold™ Kit?

A: *The two different catalog numbers are used to differentiate between the binding plates that are included in the kit. Deep and shallow-well binding plates are available to accommodate most rotors and microplate carriers. Below is a comparison of the two binding plates.*



Binding Plate	Silicon-A™ Plate	Zymo-Spin™ I-96 Plate
Style	Shallow-Well	Deep-Well
Height of Binding Plate	19 mm (0.75 inches)	35 mm (1.38 inches)
Binding Plate/Collection Plate Assembly	43 mm (1.69 inches)	60 mm (2.36 inches)
Binding Cap./Minimum Elution Volume	5 µg/30 µl	5 µg/15 µl
Catalog Numbers	D5007	D5008

Ordering Information:

Product Description	Catalog No.	Kit Size
EZ DNA Methylation-Gold™ Kit	D5005	50 rxns.
	D5006	200 rxns.
EZ-96 DNA Methylation-Gold™ Kit (Shallow-Well)	D5007	2 x 96 rxns.
EZ-96 DNA Methylation-Gold™ Kit (Deep-Well)	D5008	2 x 96 rxns.
EZ-96 DNA Methylation-Gold™ MagPrep*	D5046	4 x 96 rxns.
	D5047	8 x 96 rxns.


* **MagPrep** kits are adaptable to liquid handling robots (e.g., Tecan – Freedom EVO®) making them ideal for automated sample prep.

For Individual Sale	Catalog No.	Amount(s)
CT Conversion Reagent	D5001-1	1 tube
	D5003-1	1 bottle
M-Dilution Buffer	D5005-2	1.5 ml
	D5006-2	7 ml
M-Binding Buffer	D5005-3	30 ml
	D5006-3	125 ml
	D5040-3	250 ml
M-Wash Buffer	D5001-4	6 ml
	D5002-4	24 ml
	D5007-4	36 ml
	D5040-4	72 ml
M-Desulphonation Buffer	D5001-5	10 ml
	D5002-5	40 ml
	D5040-5	80 ml
M-Elution Buffer	D5001-6	1 ml
	D5002-6	4 ml
	D5007-6	8 ml
	D5041-6	40 ml
M-Dissolving Buffer	D5005-6	500 µl
	D5006-6	1.2 ml
Zymo-Spin™ IC Columns (capped)	C1004-50	50 columns
	C1004-250	250 columns
Collection Tubes	C1001-50	50 tubes
	C1001-500	500 tubes
	C1001-1000	1,000 tubes
MagBinding Beads	D4100-2-6	6ml
	D4100-2-8	8 ml
	D4100-2-12	12 ml
	D4100-2-16	16 ml
	D4100-2-24	24 ml
Zymo-Spin™ I-96 Binding Plates	C2004	2 plates
Silicon-A™ Binding Plates	C2001	2 plates
Conversion Plates w/ Pierceable Cover Film	C2005	2 plates/films
Collection Plates	C2002	2 plates
Elution Plates	C2003	2 plates

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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.

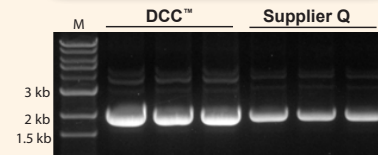
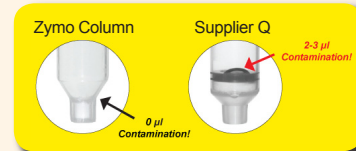


Purify DNA from PCR & other sources

DNA Clean & Concentrator™ (DCC™)

- ✓ Recovery of ultra-pure DNA that is free of salts and contaminants.
- ✓ Small ($\geq 6 \mu\text{l}$) elution volume.
- ✓ DNA is ideal for ligation, PCR, Next-Gen sequencing, etc.

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



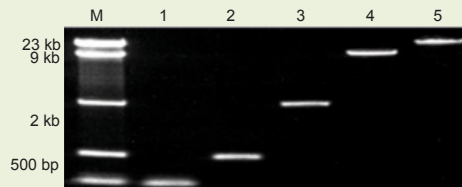
High efficiency DNA recovery with the DCC™-5 compared to Supplier Q .

Boost DNA recoveries from agarose gels to >80%

Zymoclean™ Gel DNA Recovery

- ✓ Rapid (15 min.) recovery of ultra-pure DNA from agarose gels in $\geq 6 \mu\text{l}$.
- ✓ 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) 200 Preps. (D4002)
Zymoclean™ Large Fragment DNA Recovery Kit	25 Preps. (D4045) 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)
	100 Preps. (D4019)
	400 Preps. (D4020)
	800 Preps. (D4037)



RNA 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.

Get RNA *directly* from TRIZOL® without phase separation

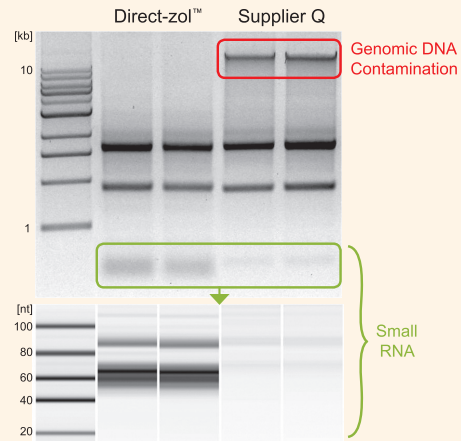
Direct-zol™ RNA

- ✓ 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)
	50 Preps. (R2051)*
	200 Preps. (R2052)
	200 Preps. (R2053)*

96-well and MagBead formats also available!

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



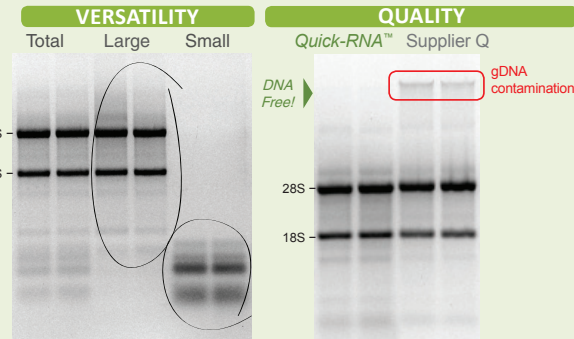
High-quality small and large RNA are effectively recovered with the Direct-zol™ kit. RNA is **DNA-free**.

Isolate DNA-free RNA from 1 to 10⁷ cells in minutes

Quick-RNA™

- ✓ Isolation of total, large, or small RNA – *You decide!*
- ✓ Ultra clean, high-quality RNA from a single cell to 10⁷ cells.
- ✓ DNA-free RNA ideal for any downstream application – *DNase I included.*

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



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

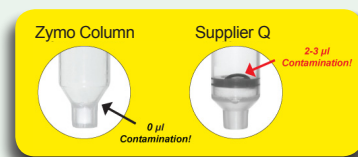
RNA is DNA-free using the Quick-RNA™ kit

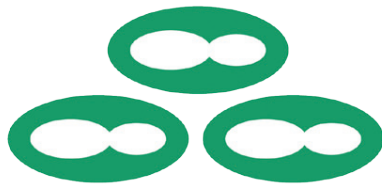
Purify RNA from enzymatic and labeling reactions in 5 minutes

RNA Clean & Concentrator™

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

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





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