Innovation to support science....

ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZR-96 Genomic DNA Clean & Concentrator™-5

Catalog Nos. **D4066 & D4067**

Highlights

- 96-well plate recovery of large-sized DNA (e.g., genomic, mitochondrial, plasmid (BAC/PAC), viral, phage, (wga)DNA, etc.) from any enzymatic reaction or impure preparation (e.g., Proteinase K digestion). *No messy precipitations!*
- Unique spin column for low volume (≥15 µl) elution of ultra-pure, high-yield DNA.
- Eluted DNA is ideal for PCR, restriction endonuclease digestion, Sanger and Next-Gen sequencing, etc.

Contents

ZYMO RESEARCH CORP.

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Satisfaction of all Zymo Research products is guaranteed. If you should be dissatisfied with this product please call 1-888-882-9682.

Product Contents

ZR-96 Genomic DNA Clean & Concentrator™-5 (Kit Size)	D4066 (2x96 Preps.)	D4067 (4x96 Preps.)	Storage Temperature
ChIP DNA Binding Buffer	100 ml	2 x 100 ml	Room Temp.
DNA Wash Buffer[1]	24 ml	48 ml	Room Temp.
DNA Elution Buffer	10 ml	16 ml	Room Temp.
Zymo-Spin™ I-96-XL Plates	2	4	Room Temp.
Collection Plates	2	4	Room Temp.
Elution Plates	2	4	Room Temp.
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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 the highest performance and reliability.

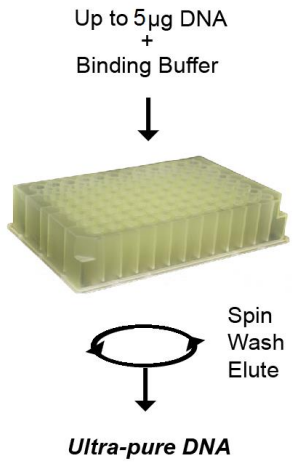
[1] Ethanol must be added prior to use as indicated on **DNA Wash Buffer** label.

Specifications

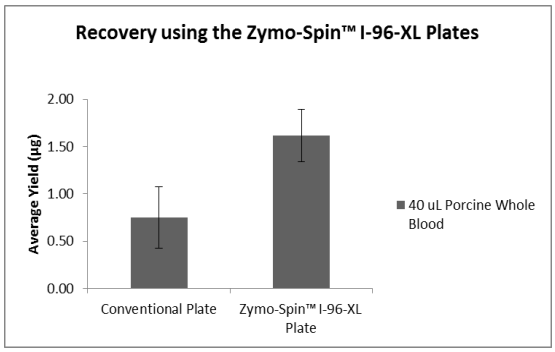
- **DNA Purity –** High-quality ($A_{(260/280)}$ ≥ 1.8) high molecular weight DNA ideal for ligation, sequencing, labeling, PCR, microarray, transfection, transformation, and restriction digestion procedures.
- **DNA Size Limits** – Capable of purifying small DNA fragments >50 bp and large sized DNAs up to 200 kb.
- **DNA Recovery** – Typically, up to 5 µg total DNA per column can be eluted into as ≥15 µl of low salt **DNA Elution Buffer** or water.
- **Sample Sources** – DNA from impure preparations of genomic DNA (e.g., Proteinase K digestions), plasmid DNA (including BAC), viral DNA, and *whole genome amplified* (wga) DNA. Can also be used for the purification of low molecular weight DNA (50 bp to 10 kb) from PCR, endonuclease digestion, post-RT cDNA synthesis, *etc.*.
- **Product Detergent Tolerance** – ≤ 5% Triton X-100, ≤ 5% Tween-20, ≤ 5% Sarkosyl, ≤ 1% SDS.

Product Description

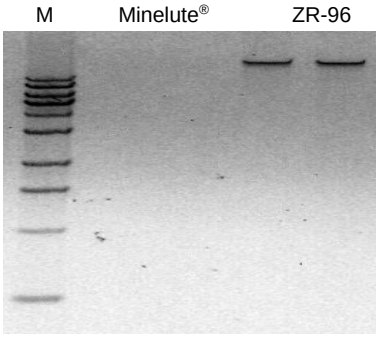
The **ZR-96 Genomic DNA Clean & Concentrator™-5 (DCC™)** is for high throughput recovery of ultra-pure, large-sized DNA (e.g., genomic, mitochondrial, plasmid (BAC/PAC), viral, phage, (wga)DNA, etc.) from any enzymatic reaction or impure preparation (e.g., Proteinase K digestion). There is no need for organic denaturants, chloroform, or messy precipitations: simply add the specially formulated **ChIP DNA Binding Buffer** to a sample and then transfer the mixture to the supplied **Zymo-Spin™ I-96-XL Plate**. Eluted DNA is suitable for sequencing, PCR, endonuclease digestion, and other enzymatic procedures. The product is also compatible with smaller DNAs (50 bp to 10 kb) from PCR, digestions, crude plasmid preparations, cDNA synthesis, etc.



Simple **ZR-96 Genomic DCC™-5** work flow.

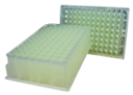


Zymo-Spin™ I-96-XL Plates result in superior yields to other conventional market columns. Genomic DNA extracted using the **Zymo-Spin™ I-96-XL Plate** results in higher yields from Porcine Whole Blood.



High molecular weight DNA is efficiently purified using the ZR-96 Genomic DCC™-25. Lambda (λ) phage DNA (48.5 kb) was purified (in duplicate) from input material using the Minelute® and the ZR-96 Genomic DCC™-5 (ZR-96). The ZR-96 Genomic DCC™-5 resulted in yields > 340% compared to the Minelute®. Eluted DNAs were analyzed in a 0.8% (w/v) TAE/agarose/EtBr gel (shown above). The size marker "M" is a 1 kb ladder (Zymo Research).

Available Formats – Genomic DNA Purification

	Genomic DCC™-10	Genomic DCC™-25	ZR-96 Genomic DCC™-5
			
Column	Zymo-Spin™ IC-XL	Zymo-Spin™ IIC-XL	Zymo-Spin™ I-96-XL
Capacity	10 µg/ prep.	25 µg/ prep.	5 µg/ prep.
Elution	≥ 10 µl	≥ 35 µl	≥ 15 µl

Available Formats – DNA Purification (< 23 kb)

	DCC™-5	DCC™-25	DCC™-100	DCC™-500	ZR-96 DCC™-5
					
Column	Zymo-Spin™ I & IC	Zymo-Spin™ II & IIC	Zymo-Spin™ V	Zymo-Spin™ VI	Zymo-Spin™ I-96
Capacity	5 µg/ prep.	25 µg/ prep.	100 µg/ prep.	500 µg/ prep.	5 µg/ prep.
Elution	≥ 6 µl	≥ 25 µl	≥ 150 µl	≥ 2 ml	≥ 10 µl

- ✓ For purification of short DNA or RNA oligonucleotides ≥16 nt, use the **Oligo Clean & Concentrator (D4060, D4061)**.
- ✓ For ChIP (Chromatin Immunoprecipitation) sample cleanup, use **the ChIP DNA Clean & Concentrator (D5201, D5205)** for high quality DNA from any step in a standard ChIP protocol.
- ✓ For post-cycle sequencing samples, use the **ZR Sequencing DNA Clean-up Kit (D4050, D4051)** for dye blob elimination.
- ✓ For samples containing PCR inhibitors, use the ***OneStep*™ PCR Inhibitor Removal Kit (D6030, D6035)**.

Typical DCC™ Applications

Post-PCR DNA Clean-up	Efficient desalting of DNA with the removal of DNA polymerases, primers and free dNTPs.
DNA Clean-up From Enzymatic Reactions	Efficient desalting of DNA with the removal of modifying enzymes, RNA polymerases, ligases, kinases, nucleases, phosphatases, endonucleases, *etc.*
Post-Reverse Transcription (RT) & cDNA Clean-up	Efficiently purifies DNA following RT, either as a DNA/RNA complex or as single stranded cDNA following chemical hydrolysis of the RNA template.
Plasmid DNA Clean-up	Efficiently purifies plasmid DNA from "home-made" preparations of cell free lysates or from commercial kits. Plasmid DNA purified and concentrated using the **DCC™** has proven an excellent substrate for high quality DNA sequencing.
Isotope and Dye Removal	Efficiently removes unincorporated fluorescent (*i.e.*, AMCA, FITC, BIO, DIG, Cy3, Cy5, FAM, *etc.*) and radiolabeled dNTP derivatives from DNA following *in vitro* labeling reactions.
Purification of M13 ssDNA	The **DCC™** can be used for the rapid isolation of single stranded M13 phage DNA directly from phage-infected *E. coli* culture supernatant.

Buffer Preparation

✓ *Before starting:* Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA Wash Buffer** concentrate. Add 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **DNA Wash Buffer** concentrate.

Protocol

Note: All centrifugation steps should be performed between 3,500 – 5,000 x g.

1. Add 2-5 volumes of **ChIP DNA Binding Buffer** to each volume of DNA sample[1] (see table below). Mix thoroughly.

Application	DNA Binding Buffer : Sample	Example
Plasmid, genomic DNA (>2 kb)	2 : 1	200 µl : 100 µl
PCR product, DNA fragment	5 : 1	500 µl : 100 µl

2. Transfer sample mixtures to the wells of the provided **Zymo-Spin™ I-96-XL Plate**[2] mounted on a **Collection Plate**.

3. Centrifuge for 5 minutes. Discard the flow through.

4. Add 200 µl **DNA Wash Buffer** to each well. Centrifuge for 5 minutes. Repeat the wash step.

5. Transfer the **Zymo-Spin™ I-96-XL Plate** to an **Elution Plate**. Add ≥ 15 µl **DNA Elution Buffer**[3] or water[4] directly to the matrix of each well and incubate at room temperature for three minutes. Centrifuge 5 minutes to elute the DNA.

   Ultra-pure DNA is now ready for use.

For **Assistance**, please contact Zymo Research Technical Support at 1-888-882-9682 or e-mail tech@zymoresearch.com.

Notes:

[1] It may be necessary to add RNase A to cell lysates prior to performing the procedure to ensure RNA-free DNA will be recovered in Step 5.

[2] The sample capacity is 900 uL/well. It may be necessary to load and spin a plate multiple times if a sample has a volume larger than 900 uL.

[3] **DNA Elution Buffer**: 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA

[4] Elution of DNA from the plate is dependent on pH and temperature. If water is used, make sure the pH is >6.0. Tthe total yield may be improved by eluting the DNA with 60-70 °C **DNA Elution Buffer**.

Troubleshooting

Low Recovery

- **Improperly Prepared/Stored DNA Wash Buffer**
  Make sure ethanol has been added to the **DNA Wash Buffer** concentrate. Cap the bottle tightly to prevent evaporation over time.
- **Addition of DNA Elution Buffer**
  Add elution buffer directly to the plate matrix and not to the walls of the plate. Elution buffer requires contact with the matrix for at least 5 minutes for large DNA ≥ 10 kb recovery.
- **Incomplete Elution**
  1. DNA elution is dependent on pH, temperature, and time. For large genomic DNA (≥ 50 kb), apply heated elution buffer (60-70 °C) and incubate for several minutes prior to elution.
  2. Sequential elutions may be performed for quantitatively higher recovery but lower final DNA concentration. This is recommended for DNA ≥ 10 kb.

Low A_{260}/A_{230} Ratios

- **Plate Tip Contaminated**
  When removing the plate from the collection plate, be careful that the tip of the plate does not come into contact with the flowthrough. Trace amounts of salt from the flowthrough can contaminate a sample resulting in low A_{260}/A_{230} ratios. Ethanol contamination from the flowthrough can also interfere with DNA elution. Zymo-Spin™ plates are designed for complete elution with no buffer retention or carryover.

Following Clean-up with the DCC™, Multiple Bands Appear in an Agarose Gel

- **Acidification of DNA Loading Dye**
  Most loading dyes do not contain EDTA and will acidify (pH ≤ 4) over time due to some microbial growth. This low pH is enough to cause DNA degradation. Therefore, if water is used to elute the DNA, 6X Loading Dye containing 1 mM EDTA is recommended.

Ordering Information

Product Description	Catalog No.	Kit Size (Preps.)
Genomic DNA Clean & Concentrator™-10 (for purification of up to 10 µg genomic DNA per prep.)	D4010 D4011	25 100
Genomic DNA Clean & Concentrator™-25 (for purification of up to 25 µg genomic DNA per prep.)	D4064 D4065	25 100
ZR-96 Genomic DNA Clean & Concentrator™-5 (for 96-well purification of up to 5 µg genomic DNA per well)	D4066 D4067	2 x 96 4 x 96

Related Products

Product Description	Catalog No.	Kit Size (Preps.)
DNA Clean & Concentrator™-5 (for purification of up to 5 µg DNA per prep.) *Supplied with **capped columns***	D4013 D4014	50 200
ZR-96 DNA Clean & Concentrator™-5 (for 96-well purification of up to 5 µg DNA per well)	D4023 D4024	2 x 96 4 x 96
DNA Clean & Concentrator™-25 (for purification of up to 25 µg DNA per prep.) *Supplied with **capped columns***	D4033 D4034	50 200
DNA Clean & Concentrator™-100 (for purification of up to 100 µg DNA per prep.)	D4029 D4030	25 50
DNA Clean & Concentrator™-500 (for purification of up to 500 µg DNA per prep.)	D4031 D4032	10 20
Oligo Clean & Concentrator™ (for purification of up to 5 µg of oligonucleotides per prep.)	D4060 D4061	50 200

For Individual Sale	Catalog No.	Size
Chip DNA Binding Buffer	D5201-1-50 D5201-1-100	50 ml 100 ml
DNA Wash Buffer (concentrate)	D4003-2-6 D4003-2-24	6 ml 24 ml
DNA Elution Buffer	D3004-4-1 D3004-4-4	1 ml 4 ml
Collection Plates	C2002	2 Plates
Elution Plates	C2003	2 Plates
Zymo-Spin™ I-96-XL Plates	C2010-2 C2010-4	2 Plates 4 Plates

DNA PURIFICATION

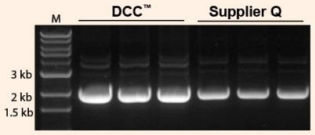
What is Clean-Spin™ Technology?
The spin columns from Zymo Research have been designed to ensure complete elution with no binding/wash buffer carryover. The result is ultra-pure inhibitor-free DNA and RNA.

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) 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)



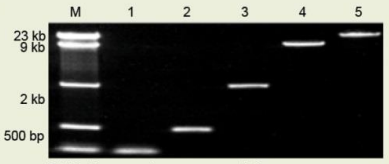
High efficiency DNA recovery with the DCC™-5 compared to Supplier Q .

Boost DNA recoveries from agarose gels to >80%

Zymoclean™ Gel DNA Recovery

- ✓ Rapid (15 min.) recovery of ultra-pure DNA from agarose gels in ≥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) 200 Preps. (D4002)
Zymoclean™ Large Fragment DNA Recovery Kit	25 Preps. (D4045) 100 Preps. (D4046)



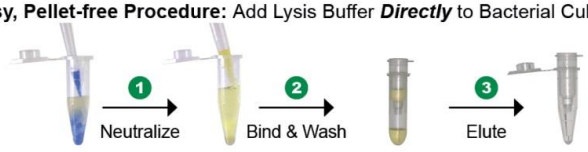
DNA fragments recovered from an agarose gel using the Zymoclean™ Gel DNA Recovery Kit. Lanes: M: DNA Ladder; 1-5: individual ladder DNA fragments.

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

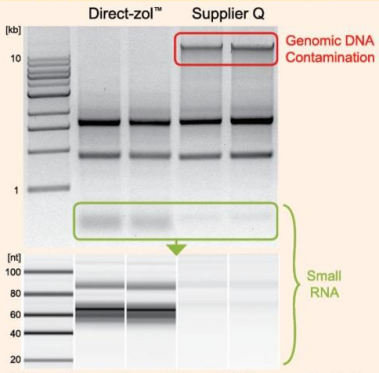
Get RNA *directly* from TRIzol® without phase separation

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®*



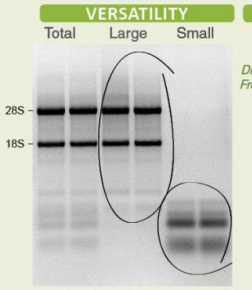
High-quality small and large RNA are effectively recovered with the Direct-zol™ kit. RNA is ***DNA-free***.

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

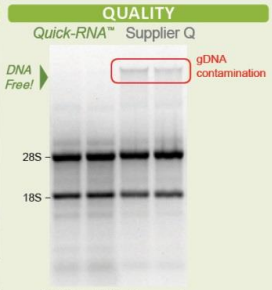
Quick-RNA™

- ✓ 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) 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, 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) 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)

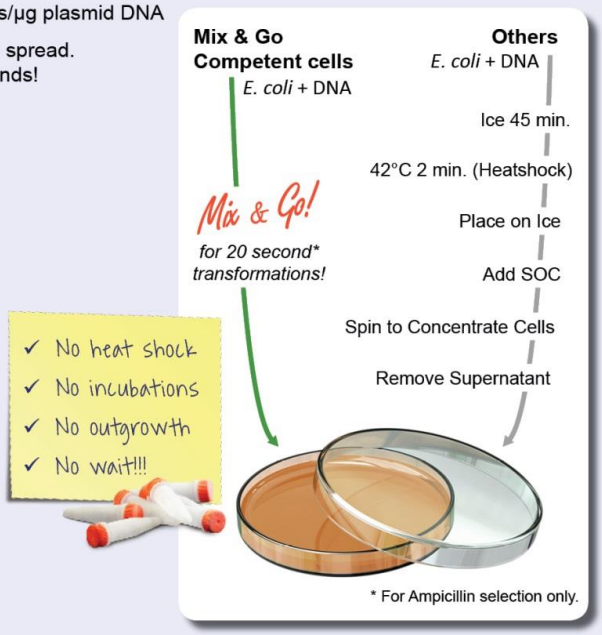
OTHER INNOVATIVE PRODUCTS FROM ZYMO RESEARCH...

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!

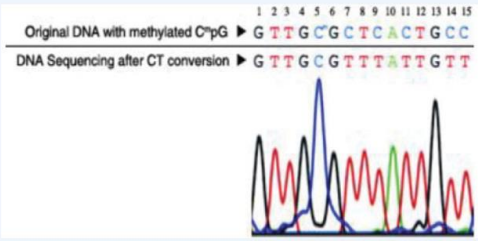
Product	Size (Cat. No.)
Zymo 5α (Same as DH5α)	10 x 100 µl aliquots (T3007) 96 x 50 µl aliquots (T3009) 96 x 50 µl aliquots PCR-plate (T3010)
Zymo 10B (Same as DH10B)	10 x 100 µl aliquots (T3019) 96 x 50 µl aliquots (T3020)
JM109	10 x 100 µl aliquots (T3003) 96 x 50 µl aliquots (T3005)
HB101	10 x 100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



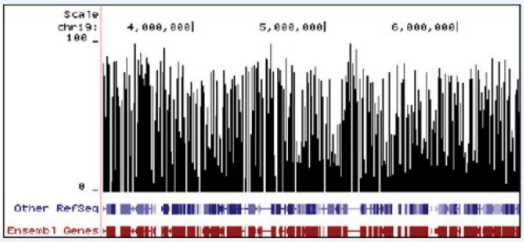
The fastest method for complete bisulfite conversion of DNA

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) 200 rxns. (D5031)
EZ-96 DNA Methylation-Lightning™ Kit	Shallow-Well Deep-Well	2 x 96 rxns. (D5032) 2 x 96 rxns. (D5033)
EZ-96 DNA Methylation-Lightning™ MagPrep		4 x 96 rxns. (D5046) 8 x 96 rxns. (D5047)