



ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

**DNA
Purification**
XXXXXXXX *Made Simple™*

INSTRUCTION MANUAL

Select-a-Size DNA Clean & Concentrator™

Catalog No. **D4080**

Highlights

- Quick and easy 7 minute protocol to select for ≥ 300 bp, ≥ 200 bp, ≥ 150 bp, ≥ 100 bp, ≥ 50 bp DNA fragments or perform a double size selection
- Clean and concentrate DNA from enzymatic reactions in as little as 10 μ l of nuclease free water
- Eluted DNA is well suited for use in next generation sequencing, PCR, or any other enzymatic reactions

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For Research Use Only

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ZYMO RESEARCH CORP.

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Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product please contact us.

Product Contents

Select-a-Size DNA Clean & Concentrator Kit	D4080 (25 Preps)	Storage Temperature
Select-a-Size DNA Binding Buffer	15 ml	Room Temp.
DNA Wash Buffer¹	6 ml	Room Temp.
DNA Elution Buffer	10 ml	Room Temp.
Zymo-Spin™ IC-S Columns (orange column)	25	Room Temp.
Zymo-Spin™ IIC Columns (clear column)	25	Room Temp.
Collection Tubes	50	Room Temp.
Instruction Manual	1	-

Note - Integrity of kit components is guaranteed for up to six months from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

Storage Temperature – Store all kit components (*i.e.*, buffers, columns) at room temperature.

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

Specifications

- **DNA Input** – up to 3 µg of double stranded DNA (dsDNA).
- **DNA Purity** – Eluted DNA is of high quality and well suited for ligations, restriction endonuclease digestions, library preparation cleanup, and next generation sequencing.
- **DNA Size Limits** – From ~50 bp to 23 kb.
- **DNA Cutoff Points** – 50 bp, 100 bp, 150 bp, 200 bp, 300 bp
Note: Alternative points can be titrated depending on the specific application (see troubleshooting).
- **Cutoff Specification** – See Table 1 on Page 2
- **Double Size Selection** – See Appendix A
- **Sample Sources** – DNA from PCR, restriction endonuclease digestions, library preparation, ligations, etc.
- **Equipment Needed** – Microcentrifuge.

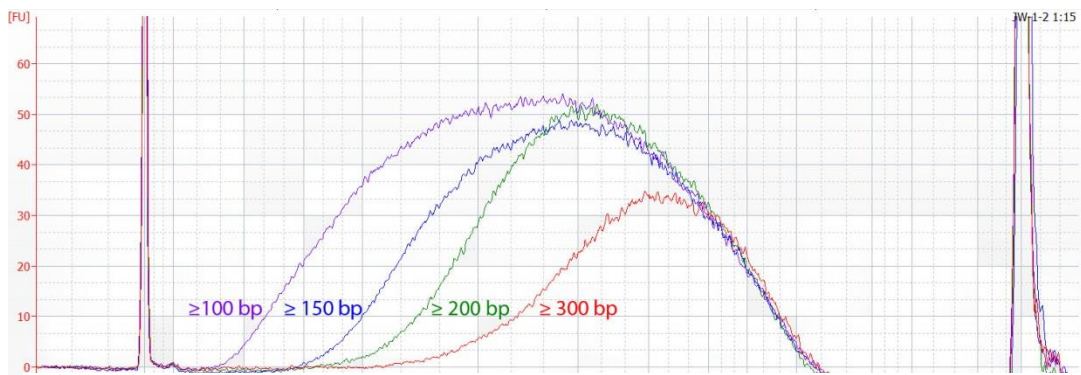
Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. It is not intended for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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Description

The **Select-a-Size DNA Clean & Concentrator™ Kit (Select-a-Size DCC™)** provides the quickest and easiest method for purifying a desired range of DNA fragments sizes from PCR, endonuclease digestions, ligations, etc. Simply adjust the binding conditions for the desired cutoff, bind, wash, and elute. Selectively recover ≥ 300 bp, ≥ 200 bp, ≥ 150 bp, ≥ 100 bp, ≥ 50 bp DNA fragments or perform a double size selection. Unique *Clean-Spin* column technology yields high-quality DNA in just minutes that is suitable for next generation sequencing¹, PCR, and other downstream applications. The entire purification procedure can be performed in as little as 7 minutes for 2 preps or 20 minutes for 24 samples. (See figures below).

¹ ChIP-seq, RNA-Seq, Methyl-Seq, DNA-Seq, etc.



Select-a-Size DNA Clean and Concentrator allows for selection at ≥ 300 bp, ≥ 200 bp, ≥ 150 bp, ≥ 100 bp and ≥ 50 bp. DNA was size selected according to the Select-a-Size DNA Clean and Concentrator protocol and the results were analyzed by Bioanalyzer. 700 ng of sonicated salmon sperm DNA was used as a standard input to evaluate size selection efficiency and cutoff. Eluted DNA was diluted 1:20 prior to being loaded on the High Sensitivity DNA Chip for analysis.



Select-a-Size DCC can be used for double size selection of samples in ranges from 50-700, 100-700, 150-700, and 200-700. The desired DNA range was selected according to the Select-a-Size DNA Clean and Concentrator protocol and the results were analyzed by Bioanalyzer. 700 ng of sonicated salmon sperm DNA, and a 70 bp amplicon was used as a standard input to evaluate size selection efficiency and cutoff. Eluted DNA was diluted 1:20 before loading onto the Bioanalyzer High Sensitivity DNA Chip for analysis.

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Notes:

¹ Cutoff is defined as the lowest detectable recovery on tapestation or Bioanalyzer instrument.

² Alternative cutoffs can be titrated using fine adjustment of volume of 95% ethanol added (see troubleshooting pg. 5).

³ For maximum enrichment, see troubleshooting section – “Choosing your cutoff.”

⁴ Nuclease-free water can be substituted for DNA Elution Buffer

⁵ To process >900 µl, reload the column.

Buffer Preparation

Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **DNA Wash Buffer**.

Protocol**Prepare size selection buffer**

Choose the desired cutoff from the table below^{1,2,3}. For complete adapter/dimer removal, select a cutoff that is at least 50 bp above the undesired fragment size (see troubleshooting section for additional details).

1. In a 1.5 mL microcentrifuge tube, add the indicated volume of **95% ethanol** followed by the **Select-a-Size DNA Binding Buffer**, mix thoroughly by pipetting the entire volume up and down 5 times:

Example: For removing dimers at 150 bp, the 200 bp cutoff should be chosen - add 500 µl Select-a-Size DNA Binding Buffer to 30 µl 95% ethanol and mix thoroughly.

DNA Fragments Retained	Volume of 95% Ethanol	Volume of <i>Select-a-Size</i> DNA Binding Buffer
≥300 bp	0 µl	500 µl
≥200 bp	30 µl	
≥150 bp	70 µl	
≥100 bp	100 µl	
≥ 50 bp	300 µl	

Perform size selection

The order of operations affects the efficiency of the size selection; therefore ensure that steps 2-4 are followed exactly (see troubleshooting for additional details).

2. In a separate tube, bring the DNA sample up to 100 µl with **DNA Elution Buffer**³.
Example: For a 20 µl sample, add 80 µl **DNA Elution Buffer** to the sample to reach a final volume of 100 µl.
3. Add DNA sample from step 2 to your binding solution from step 1. Mix thoroughly by pipetting the entire volume up and down 5 times.
4. Transfer the mixture to a **Zymo-Spin™ IC-S Column** in a **Collection Tube**⁵. Centrifuge at ≥10,000 x g for 30 seconds. Discard the flow-through.
5. Add 700 µl **DNA Wash Buffer** to the column. Centrifuge at ≥10,000 x g for 30 seconds. Discard the flow through.
6. Add 200 µl **DNA Wash Buffer** to the column. Centrifuge at ≥10,000 x g for 60 seconds. Discard the collection tube.
7. Transfer the column to a 1.5 ml microcentrifuge tube, add ≥10 µl of **DNA Elution Buffer** directly to the column matrix and incubate for 1 minute at room temperature. Centrifuge at ≥10,000 x g for 30 seconds to elute the DNA.

Ultra-pure DNA in DNA Elution Buffer is now ready for use.

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Appendix A: Double Size Selection Protocol

✓ *The order of operations affects the efficiency of the size selection; therefore ensure that steps 1-5 are followed exactly.*

Deplete the top fragments (≥700 bp)

1. Add 500 µl of **Select-a-Size DNA Binding Buffer** to 10 µl **95% Ethanol**. Mix thoroughly by pipetting up and down 5 times.
2. In a separate tube, bring the DNA sample up to 100 µl with **DNA Elution Buffer**¹.
Example: For a 20 µl sample, add 80 µl DNA Elution Buffer to the sample to reach a final volume of 100 µl.
3. Add DNA sample from step 2 to your binding solution from step 1. Mix thoroughly by pipetting the entire volume up and down 5 times.
4. Transfer the mixture to a **Zymo-Spin™ IIC Column** in a **Collection Tube**. Centrifuge at ≥10,000 x g for 30 seconds. **Save the flow-through!**

Deplete the bottom fragments (200 bp or below)

5. Choose the desired cutoff from the table below. Add the indicated volume of **95% ethanol** to the **flow-through** and mix thoroughly.

Be sure to select a cutoff at least 50 bp above your undesired DNA fragments ².

DNA Fragments Retained	Additional Volume of 95% Ethanol
≥200 bp	20 µl
≥150 bp	60 µl
≥100 bp	140 µl
≥50 bp	290 µl

6. Transfer the mixture from step 5 to a **Zymo-Spin™ IC-S Column** in a **Collection Tube**³ and centrifuge at ≥10,000 x g for 30 seconds. Discard the flow-through.
7. Add 700 µl **DNA Wash Buffer** to the column. Centrifuge at ≥10,000 x g for 30 seconds. Discard the flow through.
8. Add 200 µl **DNA Wash Buffer** to the column. Centrifuge at ≥10,000 x g for 30 seconds. Discard the collection tube.
9. Transfer the column to a 1.5 ml microcentrifuge tube, add ≥10 µl of **DNA Elution Buffer**⁴ directly to the column matrix and incubate for a minimum of 1 minute⁵. Centrifuge at ≥10,000 x g for 30 seconds to elute the DNA.

Ultra-pure DNA in DNA Elution Buffer is now ready for use.

Notes:

¹ Double Size Selection will remove DNA fragments ≥700 bp. See trouble shooting section if an alternative cutoff is required.

² See troubleshooting section on "incorrect cutoff" for additional details for selecting cutoff

³ To process >900 µl, reload the column.

Troubleshooting Guide:

Problem	Possible Causes and Suggested Solutions
Choosing your cutoff	
<p data-bbox="516 688 711 720"><i>How to choose</i></p>	<ul data-bbox="852 436 1570 913" style="list-style-type: none"> <li data-bbox="852 436 1570 562">• For maximum depletion, choose a cutoff at least 50 bp above what you are trying to remove. Choosing a cutoff around the fragment size you are trying to remove can result in recovery of undesired fragments. <li data-bbox="852 598 1570 724"><i>E.g.:</i> Choosing the 100 bp cutoff with dimers at 90 bp can result in some primer dimer/adaptor retention. The 150 bp cutoff should be minimally selected to ensure effective dimer/adaptor removal. <li data-bbox="852 756 1570 913">• Maximum enrichment is achieved when the size between cutoff and desired fragment is <u>at least</u> 100 bp. Cutoffs chosen with less than 100 bp between undesired and desired fragments can have lower recoveries around the cutoff. <p data-bbox="925 945 1570 1073"><i>E.g.:</i> If desired fragments are around 350 bp and undesired fragments are at 100 bp, choose the 150 bp cutoff for maximum retention of desired fragments while maintaining complete depletion of undesired fragments.</p>
Titrating new cutoffs	
<p data-bbox="467 1213 748 1245"><i>Single Size Selection</i></p> <p data-bbox="467 1346 748 1377"><i>Double Size Selection</i></p>	<ul data-bbox="852 1140 1570 1457" style="list-style-type: none"> <li data-bbox="852 1140 1570 1297">• Alternative cutoffs can be used by fine adjustment of the ethanol volume added to the binding buffer. A titration of the amount of ethanol used between the cutoffs may produce intermediate cutoffs that better suit a specific application. <li data-bbox="852 1329 1570 1457">• To select narrow ranges for double size selection such as 100-500 bp, ethanol can be titrated into the binding buffer prior to adding the sample, mixing, and binding onto the Zymo-Spin™ IIC column.
Incorrect cutoff point	
<p data-bbox="483 1591 771 1654"><i>Leakage of undesired fragments</i></p> <p data-bbox="495 1791 748 1822"><i>Order of operations</i></p>	<ul data-bbox="852 1524 1570 1869" style="list-style-type: none"> <li data-bbox="852 1524 1570 1661">• Ensure that you have chosen a cutoff at least 50 bp above the contaminating DNA. E.g.: To recover a library ≥ 200 bp choose the 150 bp cutoff add (70 μl 95% ethanol) to binding buffer <li data-bbox="852 1682 1570 1713">• Make sure you are using 95% ethanol <li data-bbox="852 1745 1570 1869">• Adding the binding buffer to the sample before the ethanol has been added will result in undesired cutoffs. Size selection buffer must be made prior to adding to the sample.

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Troubleshooting Guide (continued)

Problem	Possible Causes and Suggested Solutions
Low DNA quality	
<i>Poor 260/230 readings</i>	<ul style="list-style-type: none"> • Salt contamination. Be certain that both wash steps are performed. Use the DNA Wash Buffer to rinse down the walls and rim of column to remove salts. Incomplete washing will result in salt contamination (low 260/230 readings).
<i>DNA does not perform well</i>	<ul style="list-style-type: none"> • Ethanol contamination in eluate. Centrifuge the Zymo-Spin™ IC-S column in an emptied collection tube to perform a dry spin.
Double size selection	
<i>Top fragments still present after depletion</i>	<ul style="list-style-type: none"> • The Zymo-Spin™ IC-S does not efficiently deplete large DNA fragments. Be sure to use the Zymo-Spin™ IIC for efficient depletion of large DNA fragments.
Chose the wrong cutoff	
<i>How to recover your DNA</i>	<ul style="list-style-type: none"> • <i>SAVE THE FLOW THROUGH!</i> Add 95% ethanol to the <u>flow-through</u> so the final volume of ethanol within the mixture is 300 µl, mix thoroughly before transferring back to the <u>same Zymo-Spin™ IC-S</u> previously used. Bind, wash and elute the DNA according to the protocol. The eluted DNA is ready to be size selected again using the correct cutoff.
Increasing Recovery	
<i>Increase incubation time</i>	<ul style="list-style-type: none"> • Elution of DNA from the column is dependent on pH and temperature. If water is used, make sure the pH is >5.0 for optimal recovery. Waiting 5 minutes after adding water to the column may improve the yield DNA.
DNA Elution Buffer	
	<ul style="list-style-type: none"> • DNA Elution Buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.5)

Troubleshooting Guide (continued)

Problem	Possible Causes and Suggested Solutions
Low DNA Quality	
<i>Wash buffer</i>	<ul style="list-style-type: none">• Ensure that ethanol has been added to the DNA Wash Buffer.• Ensure that the bottle cap is screwed on tightly after each use to prevent evaporation.
<i>DNA elution</i>	<ul style="list-style-type: none">• Incomplete elution: For DNA fragments over 5 kb, heat the DNA Elution Buffer at 50°C and incubate the column for 5-10 minutes before centrifugation for higher recovery

Ordering Information

Product Description	Kit Size	Catalog No.
<i>Select-a-Size</i> DNA Clean & Concentrator™	25 Preps	D4080

For Individual Sale	Amount	Catalog No.
<i>Select-a-Size</i> DNA Binding Buffer	15 ml	D4080-1-15
Zymo-Spin™ IC-S Column	25 Columns	C1015-25
Zymo-Spin™ IIC Column	50 Columns	C1011-50
DNA Wash Buffer	6 ml	D4003-2-6
DNA Elution Buffer	10 ml	D3004-4-10
Collection Tubes	50	C1001-50

Note - Integrity of kit components is guaranteed for up to **6 months** from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability. - ™ 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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