ZYMO RESEARCH

DNA Purification Made Simple

Select-a-Size DNA Clean & Concentrator®

Purify desired range of DNA fragments sizes from library preps, PCR, endonuclease digestions, ligations, etc.

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 µl of nuclease free water.
- Eluted DNA is well suited for use in next generation sequencing, PCR, or any other enzymatic reactions.

Catalog Numbers:
D4080

Scan with your smart-phone camera to view the online protocol/video.

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682

Table of Contents

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 Buffer[1]	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	-

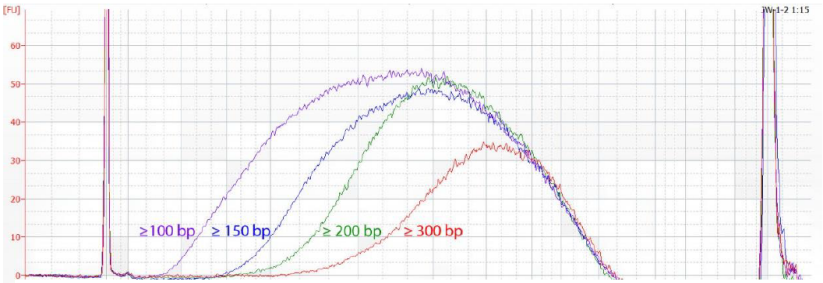
[1] Ethanol must be added prior to use as indicated on **DNA Wash Buffer** label.

Specifications

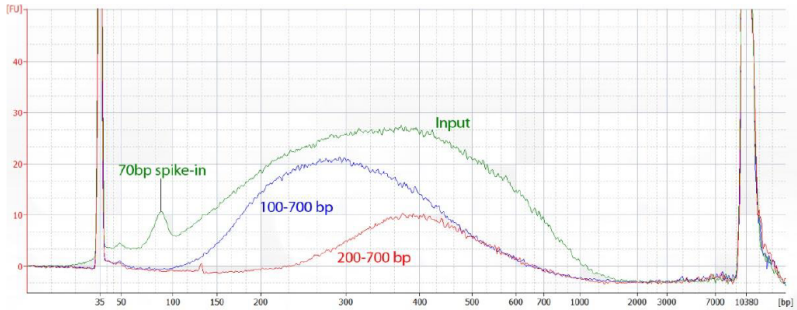
- **DNA Input –** up to 3 µg of double stranded DNA (dsDNA).
- **DNA Purity –** Eluted DNA is of high quality and well suited for ligations, restriction endonuclease digestions, library preparation cleanup, and next generation sequencing.
- **DNA Size Limits –** From ~50 bp to 23 kb.
- **DNA Cutoff Points –** 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 on Page 4
- **Double Size Selection –** See Protocol on page 5
- **Sample Sources –** DNA from PCR, restriction endonuclease digestions, library preparation, ligations, etc.
- **Equipment Needed –** Microcentrifuge.

Product 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 *Fast Spin* column technology yields high-quality DNA in just minutes that is suitable for next generation sequencing[1], 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).



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.

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

Protocol

Buffer Preparation

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

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 *Select-a-Size* 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*[4]**.

   ***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.

---

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. 7).
3 For maximum enrichment, see troubleshooting section – "Choosing your cutoff."
4 Nuclease-free water can be substituted for **DNA Elution Buffer**.

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.

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**[6].

   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!**

---

5 To process >900 µl, reload the column.
6 Double Size Selection will remove DNA fragments ≥700 bp. See troubleshooting section if an alternative cutoff is required.

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[7]*.*

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**[8] 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** directly to the column matrix and incubate for a minimum of 1 minute. 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.

7 See troubleshooting section on "incorrect cutoff" for additional details for selecting cutoff.
8 To process >900 µl, reload the column.

Troubleshooting

Problem	Possible Causes and Suggested Solutions
Choosing Your Cutoff	
	• 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 **<u>can</u>** result in recovery of undesired fragments. ***E.g.*: *Choosing the 100 bp cutoff with dimers at 90 bp can result in some primer dimer/adapter retention. The 150 bp cutoff should be minimally selected to ensure effective dimer/adapter removal.*
How to choose	• 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. ***E.g.*: *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.*
Titrating New Cutoffs	
Single Size Selection	• 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 **<u>may</u>** produce intermediate cutoffs that better suit a specific application.
Double Size Selection	• 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	
Leakage of undesired fragments	• Ensure that you have chosen a cutoff at **<u>least</u>** 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. • Make sure you are using 95% ethanol.

Order of operations	• 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.
Low DNA Quality	
Poor 260/230 readings	• **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).
DNA does not perform well	• **Ethanol contamination in eluate**. Centrifuge the **Zymo-Spin™ IC-S Column** in an emptied collection tube to perform a dry spin.
Wash buffer	• 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.
DNA elution	• **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
Double Size Selection	
Top fragments still present after depletion	• The **Zymo-Spin™ IC-S Column** does not efficiently deplete large DNA fragments. Be sure to use the **Zymo-Spin™ IIC Column** for efficient depletion of large DNA fragments.
Chose the Wrong Cutoff	
How to recover your DNA	• ***SAVE THE FLOW THROUGH!*** Add 95% ethanol to the flow-through so the final volume of ethanol within the mixture is 300 µl, mix thoroughly before transferring back to the same **Zymo-Spin™ IC-S Column** 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	
Increase incubation time	• 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	
	• DNA Elution Buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.5)

Ordering Information

Product Description	Catalog No.	Size
Select-a-Size DNA Clean & Concentrator®	D4080	25 Preps.

Individual Kit Components	Catalog No.	Amount
***Select-a-Size* DNA Binding Buffer**	D4080-1-15	15 ml
DNA Wash Buffer	D4003-2-6	6 ml
DNA Elution Buffer	D3004-4-10	10 ml
Zymo-Spin™ IC-S Column	C1015-25	25 Pack
Zymo-Spin™ IIC Column	C1011-50	50 Pack
Collection Tubes	C1001-50	50 Pack

100% satisfaction guarantee on all Zymo Research products, or your money back.

Zymo Research is committed to simplifying your research with quality products and services. If you are dissatisfied with this product for any reason, please call 1(888) 882-9682.

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 the highest performance and reliability.

This product is for research use only and should only be used by trained professionals. It is not 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.

ZYMO RESEARCH

The **BEAUTY** *of* **SCIENCE** *is to Make Things* **SIMPLE**®

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682