AdvancedSeq LLC,
Your Reliable Sanger Sequencing Reagents Partner

Make Your Sanger Sequencing Cost Effective

High-Quality Products for Sanger Sequencing

- WORLDWIDE ADOPTION
- HIGH-QUALITY ASSURANCE
- CUSTOMER-APPROVED PERFORMANCE

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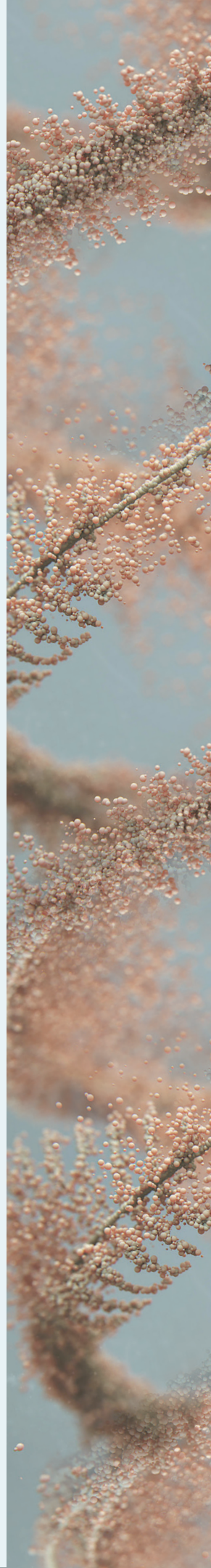
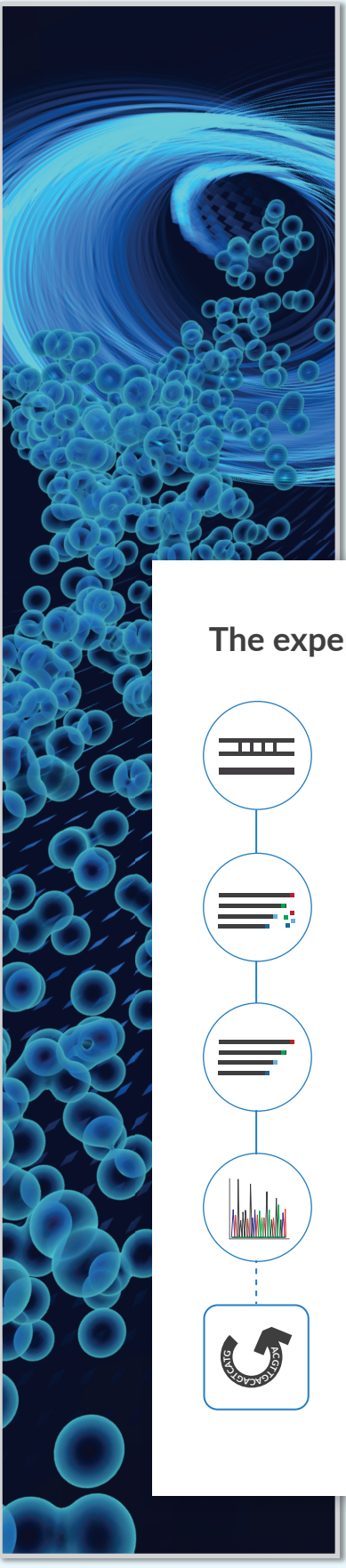


Products at a Glance

Workflow Steps	Product Name	Unit Size	Catalog #
Cycle Sequencing Reactions	SupreDye™ BD1 Cycle Sequencing Kits	100 rxn	060002
		2500 rxn	060020
		5000 rxn	060040
	SupreDye™ BD3 Cycle Sequencing Kits	100 rxn	063002
		2500 rxn	063020
		5000 rxn	063040
	SupreDye™ dGTP BD1 Cycle Sequencing Kits	100 rxn	061002
		2500 rxn	061020
	SupreDye™ dGTP BD3 Cycle Sequencing Kits	100 rxn	065002
		2500 rxn	065020
	ADS™ 5x Sequencing Buffer	28 ml	070028
		233 ml	070233
Sequencing Cleanup	ADS™ BD-XT Purification Kits	100 preps	160001
		1000 preps	160010
		2500 preps	160025
	ADS™ Sequencing Reaction Cleaning Beads	50 ml	080050
		200 ml	080250
Sample Loading and Capillary Electrophoresis	TruPure™ Formamide	25 ml	090025
		250 ml	090250
	PwrPOP™ P7 Sequencing Polymer	7 ml	037007
		28 ml	037010
	PwrPOP™ P6 Sequencing Polymer	7 ml	036007
		28 ml	036010
	PwrPOP™ P4 Sequencing Polymer	7 ml	034007
		28 ml	034010
	ADS™ Conformational Analysis Polymer (CAP)	25 ml	032025
	ADS™ 10x Sequencing Running Buffer	500 ml	010500
Regeneration of Capillary Array	ADS™ Capillary Regeneration Kits	4x7 ml	150007
		4x28 ml	150028
Template Preparation	ADS™ PCR Cleaning Magnetic Beads	1 ml	170001
		5 ml	170005
	ADS ™ Taq DNA Polymerase	400 units, 80 µl, 1.25 U/50 ul Rxn	101004
		2000 units, 400 ul, 1.25 U/50 ul Rxn	101020
	ADS™ PCR and Purification kits	Taq polymerase 40 units, 8 µl; 10 mM dNTPS, 40 ul; 5X PCR green buffer, 400 µl; PCR cleaning beads, 2 ml;	101000K
	dNTPs Mix	10 mM each, 1 ml	100401DNS
		10 mM each, 5 ml	100405DNS
	5xPCR Green Buffer	1 ml	041001
		25 ml	041025



SANGER SEQUENCING WORKFLOW

Dideoxy chain-termination Sanger sequencing has been used in the past 40 years as a gold standard for gene mutation analysis, de novo sequencing, resequencing, confirmation of next-generation sequencing (NGS), and gap filling for NGS. As a mature technology, Sanger sequencing will continue to play an important role in the DNA sequencing field.

The experimental workflow for Sanger Sequencing Includes:

STEP 1. Template Preparation

Preparation of high-quality sequencing templates and primers for setting up sequencing reactions. PCR templates need to be purified or cleaned up to remove dNTPS, primers and DNA polymerase.

STEP 2. Cycle Sequencing Reaction Set-up

Use of one primer and one template to linearly amplify and generate nested fluorescently labeled extension products with a single nucleotide difference.

STEP 3. Sequencing Cleanup

Cleanup of completed sequencing reactions to remove unincorporated dyes and other reaction components from the end-labeled extension products.

STEP 4. Sample Loading and Capillary Electrophoresis (CE)

Loading extension products with or without resuspension on CE instruments for detecting fluorescent signals, which are converted into DNA sequence using a base calling software.

STEP 5. Regeneration of Capillary Array

As a supplementary workflow step for instrument maintenance, regeneration of the capillary array to remove contaminants accumulated on the array after hundreds of CE runs. Regenerated array regains optimal performance.

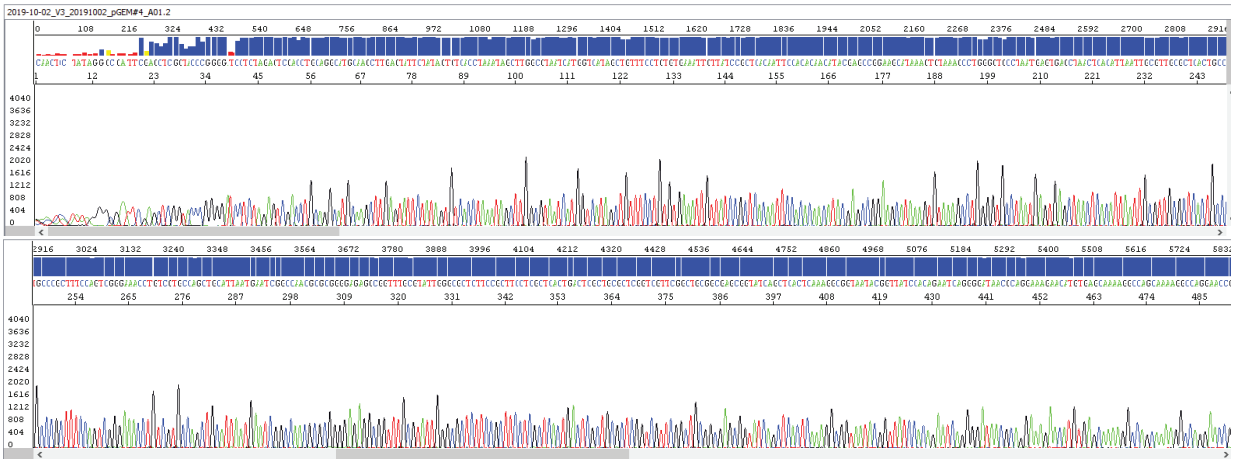
PRODUCT OFFERINGS

AdvancedSeq LLC specializes in making high-quality DNA sequencing (especially Sanger sequencing) and PCR reagents for life science research at competitive prices. Founded by scientists and product managers with extensive knowledge and experience in the life science industry, the company produces products that are trusted and used by many companies and academic labs worldwide. We have distributors and sales force in the US, Europe and China, and are fully committed to providing high-quality, cost-effective products to our customers with superior technical support.

For Sanger sequencing, we provide products used in all steps of the workflow. The featured products listed below have proven performance and are offered as alternatives for equivalent products used to carry out a daily workflow of a sequencing facility.

FEATURED PRODUCTS

- SupreDye™ Cycle Sequencing Kits
- PwrPOP™ Polymers
- ADS™ BD-XT Purification Kits
- TruPure™ Formamide
- ADS™ Capillary Regeneration Kits



Reliable high-quality system performance: all the sequencing reagents used were from Advancedseq (SupreDye™ Cycle Sequencing Kit, ADS™ Sequencing Reaction Cleaning Beads, and PwrPOP™ P7 Polymer). A sequencing sample was run on a 3130xl Genetic Analyzer and data were analyzed using the Sequencing Analysis v5.4 software.

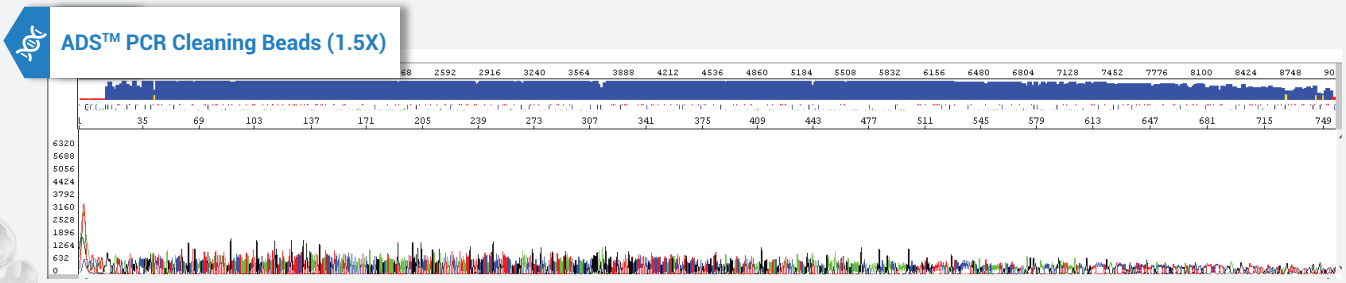
TEMPLATE PREPARATION

Preparing high-quality sequencing templates is one of the key parameters for sequencing success. Among the various sequencing template types, PCR templates, along with plasmid templates, are the most popular and need careful preparation for optimal sequencing quality.

For PCR templates, conditions should be optimized with high-grade reagents to get a single PCR product so the purification step to remove other non-specific PCR products can be eliminated. Purification of the PCR product from other PCR components, such as primers, dNTPS, and DNA polymerase, is needed. The residual contaminants may cause suboptimal sequencing reactions by changing the reaction conditions or by interfering with the reaction.

Although there are other PCR product cleaning methods, spin column purification and magnetic bead purification are the most common PCR cleanup methods. Of these, spin column purification is more expensive and more time-consuming if high-throughput purification is needed.

Finally, all types of sequencing-ready purified templates must be quantified and the right amount of DNA template should be used for sequencing reactions according to the sequencing guide.



Electropherogram data from Sanger sequencing of a 1.2 kb PCR fragment using ADS™ PCR Cleaning Beads at 1.5 volume of the PCR reaction volume.

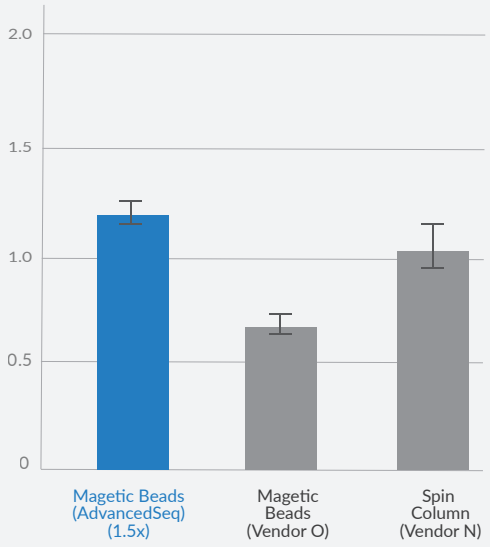
ADS™ PCR Cleaning Beads

PRODUCT IMAGE

PRODUCT KEY HIGHLIGHT

- Adaptable with high-throughput template preparation
- Compatible with DNA sequencing workflow
- High DNA recovery
- Uniform bead suspension

SUPPORTING DATA



High DNA recovery using AdvancedSeq magnetic beads. Products used from different vendors for PCR product purification.

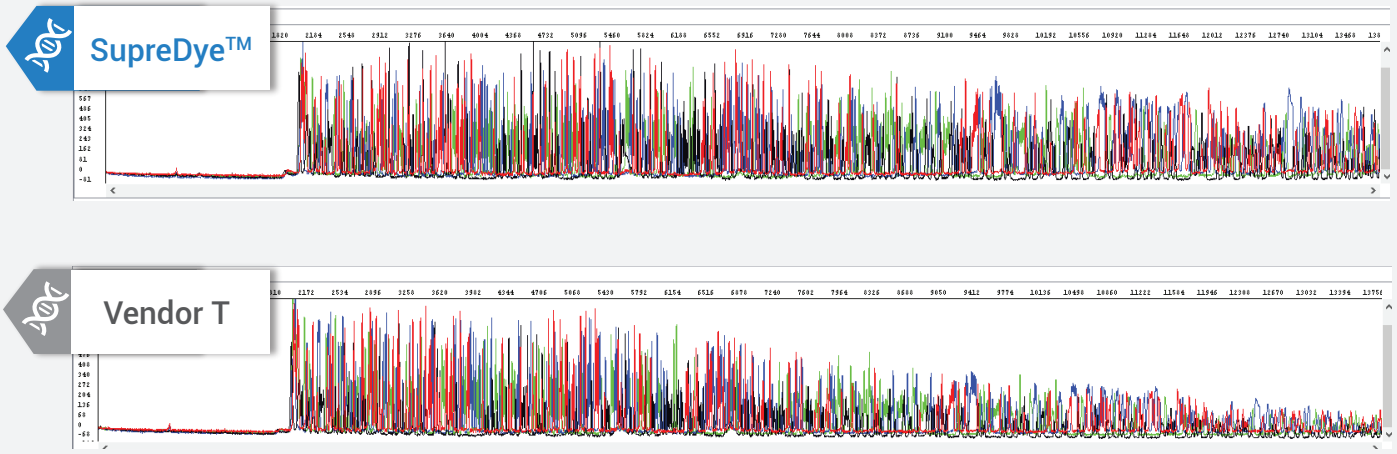
ORDERING INFORMATION

Product Name	Unit Size	Catalog #
ADS™ PCR Cleaning Magnetic Beads	1 ml	170001
	5 ml	170005
ADS™ Taq DNA Polymerase	400 units, 80 µl, 1.25 U/50 ul Rxn	101004
	2000 units, 400 ul, 1.25 U/50 ul Rxn	101020
ADS™ PCR and Purification kit	Taq polymerase 40 units, 8 µl; 10 mM dNTPS 40 ul; 5X PCR green buffer, 400 µl; PCR cleaning beads, 2 ml	101000K
dATP Nucleotides	100 mM, 200 µl	100020DA
	100 mM, 1 ml	100100DA
dCTP Nucleotides	100 mM, 200 µl	100020DC
	100 mM, 1 ml	100100DC
dGTP Nucleotides	100 mM, 200 µl	100020DG
	100 mM, 1 ml	100100DG
dTTP Nucleotides	100 mM, 200 µl	100020DT
	100 mM, 1 ml	100100DT
dNTPs Mix	10 mM each, 1 ml	100401DNS
	10 mM each, 5 ml	100405DNS
A Set of 4 Nucleotides (dATP, dCTP, dGTP, and dTTP)	100mM each, 4x200 µl	100002DN
	100mM each, 4x1 ml	100010DN
5xPCR Green Buffer	1 ml	041001
	25 ml	041025

CYCLE SEQUENCING REACTIONS

Cycle sequencing reactions use linear amplification to produce end-labeled extension products based on one primer and one template in a reaction. A nest of extension products with only one nucleotide difference is made due to chain-termination resulting from incorporation of fluorescent dideoxynucleotides (ddNTPs).

In regular cycle sequencing kits, dITP is used to replace dGTP to reduce peak compression in CE. However, for high G- templates (GT or GC), dITP in a regular kit is replaced with dGTP to optimize sequencing performance.



SupreDye™ Cycle Sequencing Kit (top panel) shows much better signal intensity and stability compared to the Vendor T product (bottom panel).

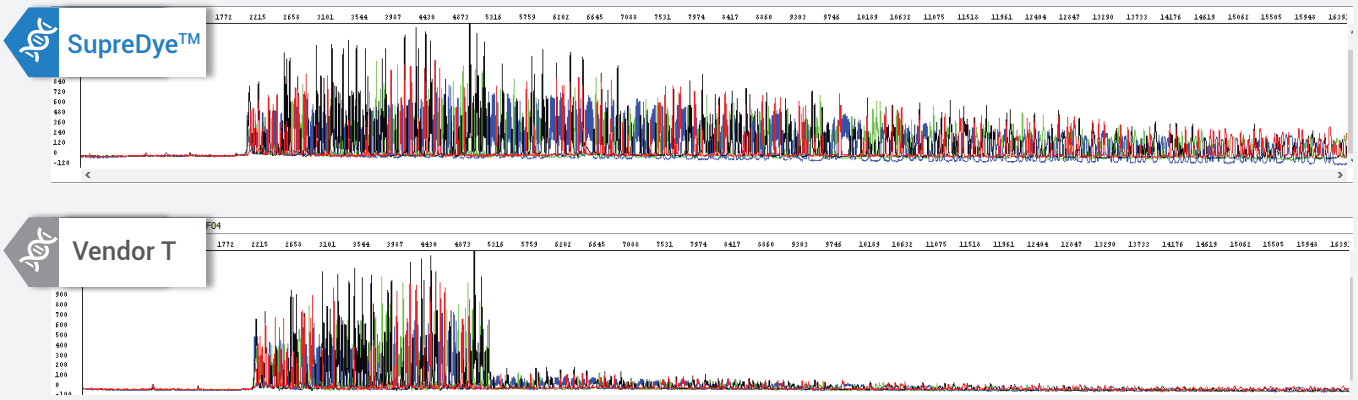
SupreDye™ Cycle Sequencing Kits

PRODUCT IMAGE

PRODUCT KEY HIGHLIGHT

- Proven high-performance
- Broadly accepted in community
- Increased robustness for difficult templates
- Even peak heights and long read lengths

SUPPORTING DATA



Compared to the Vendor T product (bottom panel), SupreDye™ chemistry (top panel) shows much better performance for sequencing a difficult template with secondary structures by generating strong and long-read signal.

ORDERING INFORMATION

Product Name	Unit Size	Catalog #
SupreDye™ BD1 Cycle Sequencing Kit	100 rxn	060002
	2500 rxn	060020
	5000 rxn	060040
SupreDye™ BD3 Cycle Sequencing Kit	100 rxn	063002
	2500 rxn	063020
	5000 rxn	063040

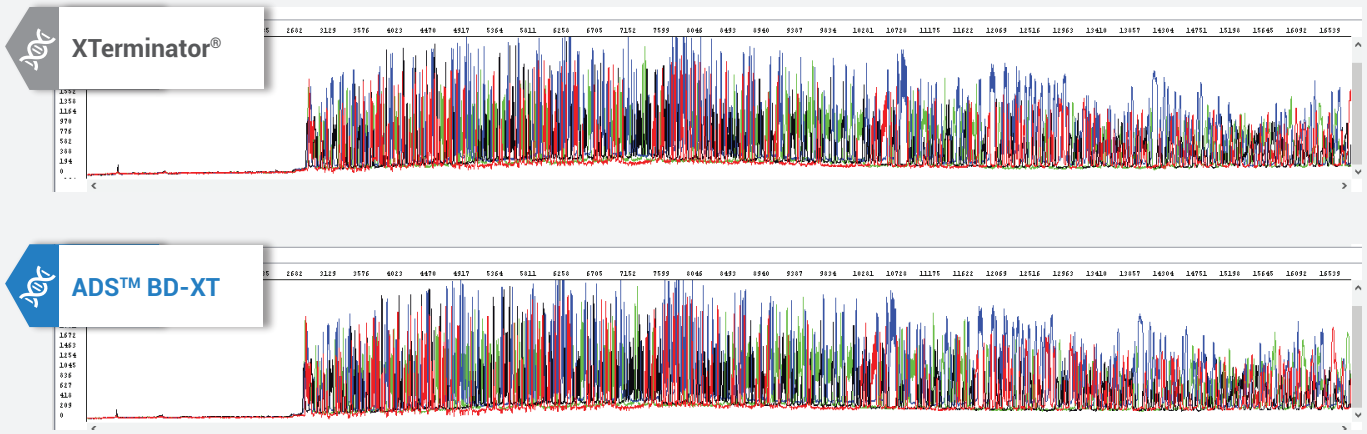
Product Name	Unit Size	Catalog #
SupreDye™ dGTP BD1 Cycle Sequencing Kit	100 rxn	061002
	2500 rxn	061020
SupreDye™ dGTP BD3 Cycle Sequencing Kit	100 rxn	065002
	2500 rxn	065020
ADS™ 5x Sequencing Buffer	28 ml	070028
	233 ml	070233

SEQUENCING CLEANUP

It is essential to remove the contaminants, especially the unincorporated ddNTP dyes, from the completed sequencing reaction. Failure to remove these contaminants results in signal reduction and interference of peak separation and reading.

Different mechanisms are available for cleaning up the sequencing reactions. In one mechanism, contaminants bind to special resins and get removed. In another mechanism, extension products bind to magnetic beads and are separated from contaminants. Contaminants can also be removed by ethanol precipitation of DNA, a time-consuming procedure. It is important to compare the performance of different purification kits in terms of downstream sequencing quality.

We provide ADS™ BD-XT Purification Kit and ADS™ Sequencing Reaction Cleaning Beads, both of which are optimized for downstream sequencing performance.



Comparable sequencing performance from samples purified using Thermo Fisher Scientific Xterminator® Purification Kit (top panel) and AdvancedSeq ADS™ BD-XT Purification Kit (bottom panel).

ADS™ BD-XT Purification Kits

PRODUCT IMAGE AND KEY HIGHLIGHTS

- Fast, reliable, reproducible
- Complete dye blob removal
- Simple workflow, minimal hands-on time
- Excellent short and long fragment recovery, higher signal

ORDERING INFORMATION

Product Name	Unit Size	Catalog #
ADS™ BD-XT Purification Kit	100 preps	160001
	1000 preps	160010
	2500 preps	160025

ADS™ Sequencing Reaction Cleaning Beads

PRODUCT IMAGE AND KEY HIGHLIGHTS

- Specially optimized for DNA sequencing
- Ensuring product integrity and high recovery
- Minimal wash steps and simple workflow
- Cost-effective cleaning

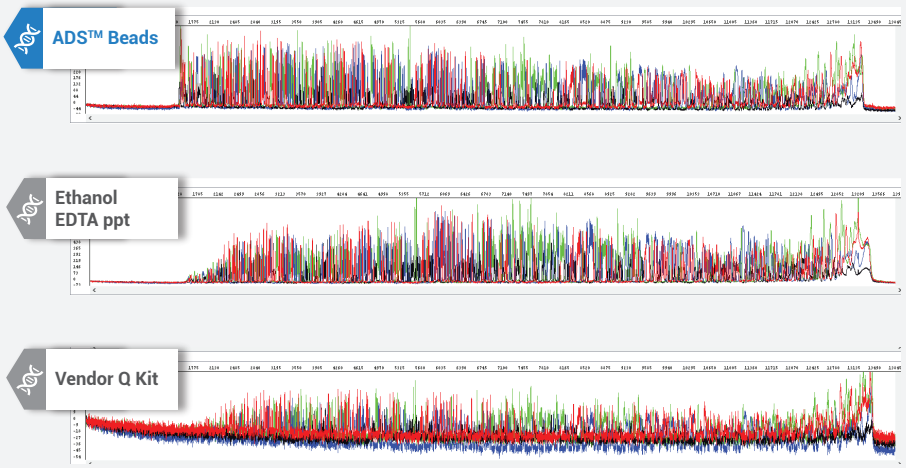
ORDERING INFORMATION

Product Name	Unit Size	Catalog #
ADS™ Sequencing Reaction Cleaning Beads	50 ml	080050
	200 ml	080250

Choosing the Right Kit for Sequencing Reaction Cleanup

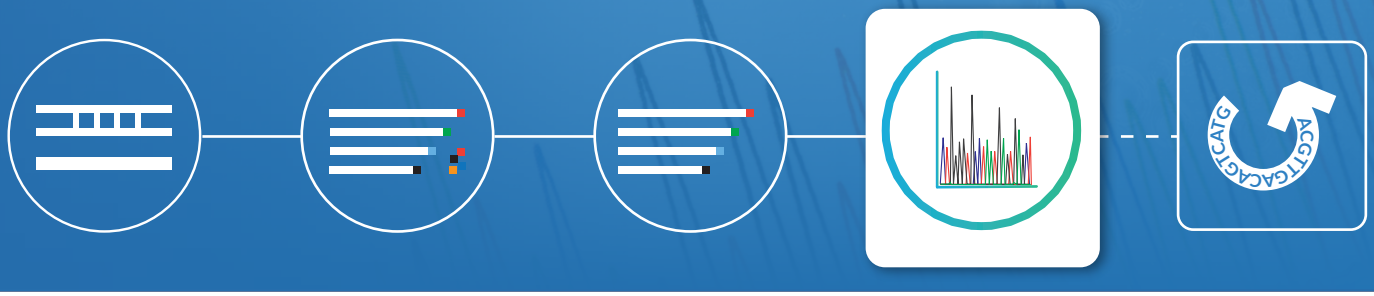
Both kits can effectively remove sequencing contaminants and achieve desired sequencing results. With ADS™ BD-XT Purification Kit, the washing steps, which are part of the protocol for ADS™ Sequencing Reaction Cleaning Beads, can be eliminated. The purified products can then be directly loaded to the sequencer without sample resuspension, which is also necessary for ADS™ Sequencing Reaction Cleaning Beads. The ADS™ Sequencing Reaction Cleaning Beads are more cost-effective; compared to other magnetic beads used for the same application, the ADS™ beads are optimized for achieving high sequencing quality with fewer washes but with less dye-blobs.

SUPPORTING DATA



Sequencing raw data comparison for the same sequencing reaction purified by ADS™ Beads (top panel), ethanol purification (middle panel), and Vendor Q kit spin column purification (bottom panel). Both ethanol precipitation and Vendor Q kit lose small extension products and generate significantly reduced signals near the primer site.

SAMPLE LOADING AND CAPILLARY ELECTROPHORESIS



After sequencing reactions are cleaned up or purified, they are ready to be loaded on genetic analyzers for capillary electrophoresis. Depending on the purification methods or products used, the samples may be directly loaded to CE or need to be resuspended either in water/buffer or formamide before loading. Sequencing reactions purified by ethanol precipitation are often resuspended in highly deionized formamide for optimal signal intensity and stability.

Polymers as separation matrices are important for effective separation of extension products. It is critical to use high-quality polymer products to get traces with good signal intensity, even peak heights and spaces for accurate basecalls and long reads.

PwrPOP™ P7 Polymer (bottom panel) shows comparative separation performance with Polymer from Vendor T (top panel).

TruPure™ Formamide

PRODUCT IMAGE

PRODUCT KEY HIGHLIGHT

- High purity
- High signal intensity
- Great signal stability

PwrPOP™ Polymers

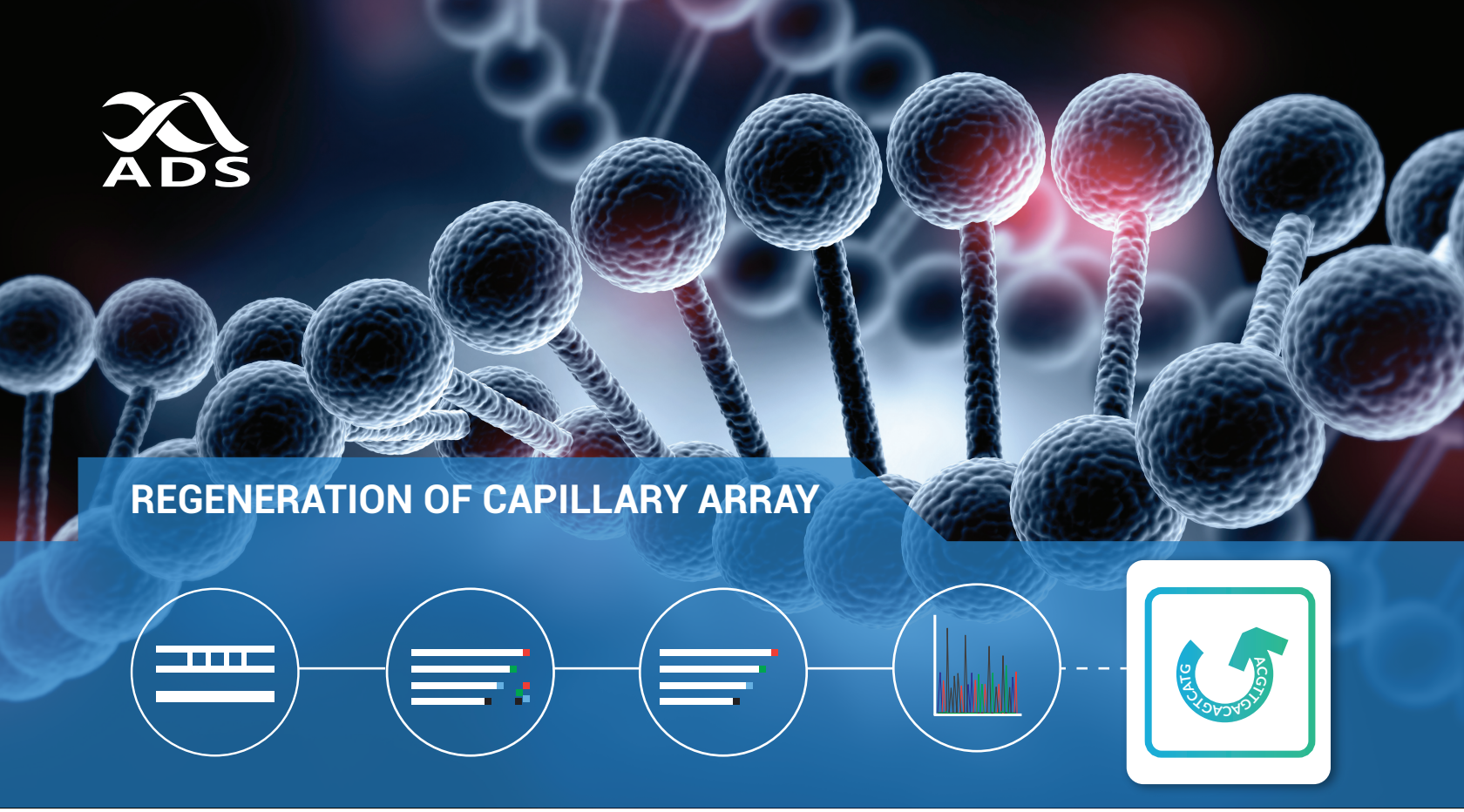
PRODUCT IMAGE

PRODUCT KEY HIGHLIGHT

- Optimized for DNA sequencing and fragment analysis
- Even peak separation for long read
- Cost-effective

ORDERING INFORMATION

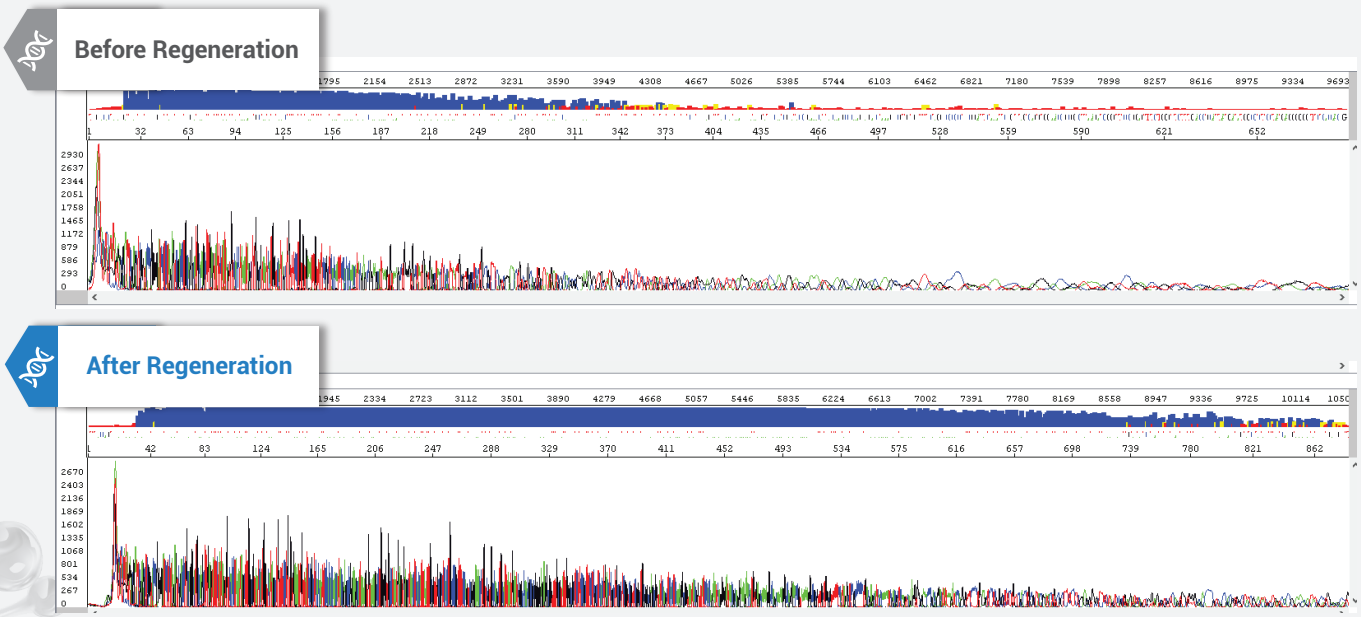
Product Name	Unit Size	Catalog #
TruPure™ Formamide	25 ml	090025
	250 ml	090250
PwrPOP™ P7 Sequencing Polymer	7 ml	037007
	28 ml	037010
PwrPOP™ P6 Sequencing Polymer	7 ml	036007
	28 ml	036010
PwrPOP™ P4 Sequencing Polymer	7 ml	034007
	28 ml	034010
ADS™ Conformational Analysis Polymer (CAP)	25 ml	032025
ADS™ 10x Sequencing Running Buffer	500 ml	010500



REGENERATION OF CAPILLARY ARRAY

After extensive usage, capillary arrays need to be regenerated to remove debris accumulated inside the capillaries. This helps restore normal performance in the separation of extension products. Without proper regeneration, the lifetime of the capillary array can be short and the replacement cost becomes significant.

Experienced users are able to recognize the appropriate time to regenerate the capillary array based on substandard array performance such as the uneven peak space and shortened reading length. Once the array is regenerated, it will return to its optimal operating conditions. We recommend ADS™ Capillary Regeneration Kit be used after every 500 runs to keep the performance of the capillaries optimal.



ADS™ Capillary Array Regeneration Kit provides much better signal intensity and read length (bottom panel) compared to data prior to regeneration (top panel).

ADS™ Capillary Regeneration Kits

PRODUCT IMAGE

PRODUCT KEY HIGHLIGHT

- Easy implementation
- On instrument protocol
- No capillary assembly/disassembly
- Short hands-on time
- Cost-effective

ORDERING INFORMATION

Product Name	Unit Size	Catalog #
ADS™ Capillary Regeneration Kit	4x7 ml	150007
	4x28 ml	150028

AdvancedSeq provides high-quality Sanger sequencing products distributed worldwide. We suggest that you join our community to be the first to get our future new products information and test them.

Here is how you can purchase our products:

- By Phone: call us at 818-639-4362
- Online: purchase product directly online (www.advancedseq.com) once you register an account with us.

Your local distributor:

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