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

Stratech

stratech.co.uk/advancedseq

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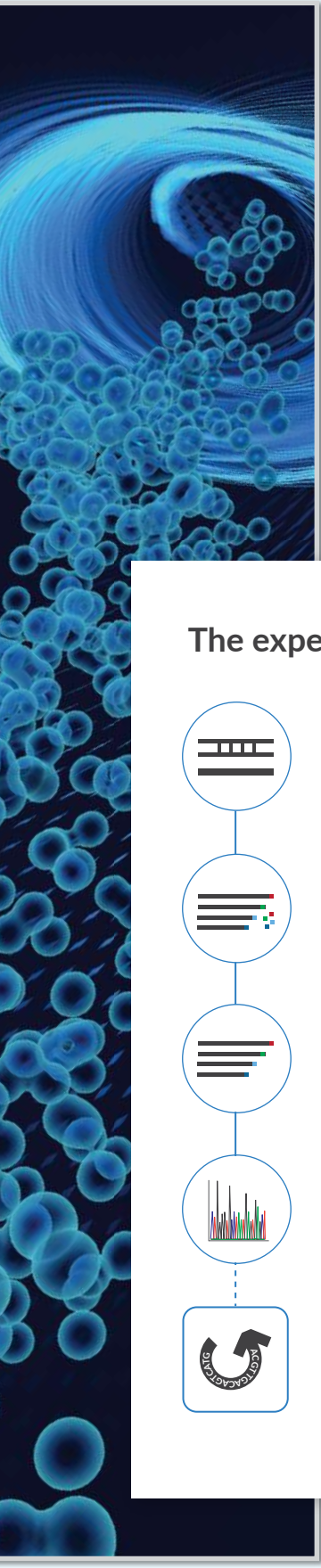
# Products at a Glance

| 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;<br>10 mM dNTPS, 40 ul;<br>5X PCR green buffer, 400 µl;<br>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.

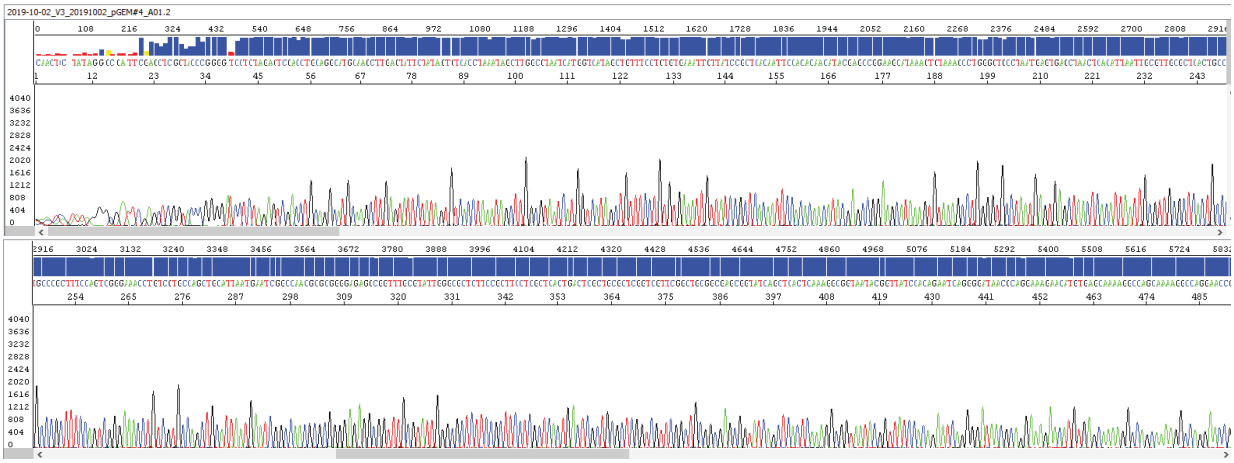
# PRODUCT OFFERINGS

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.

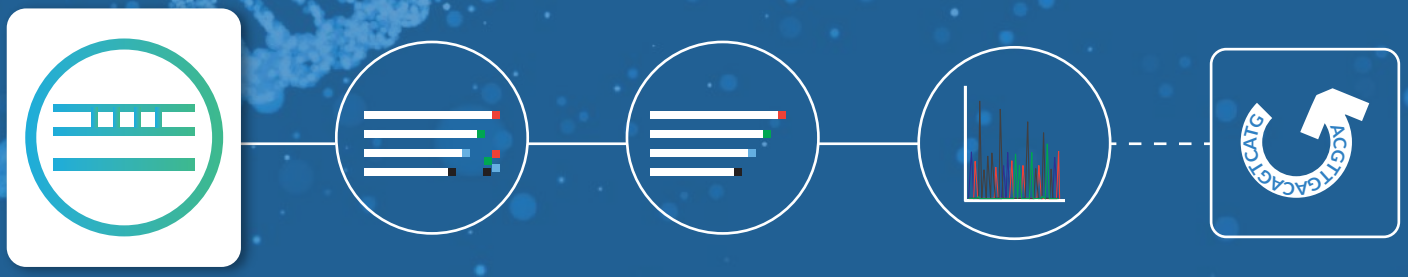
## FEATURED PRODUCTS

- 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

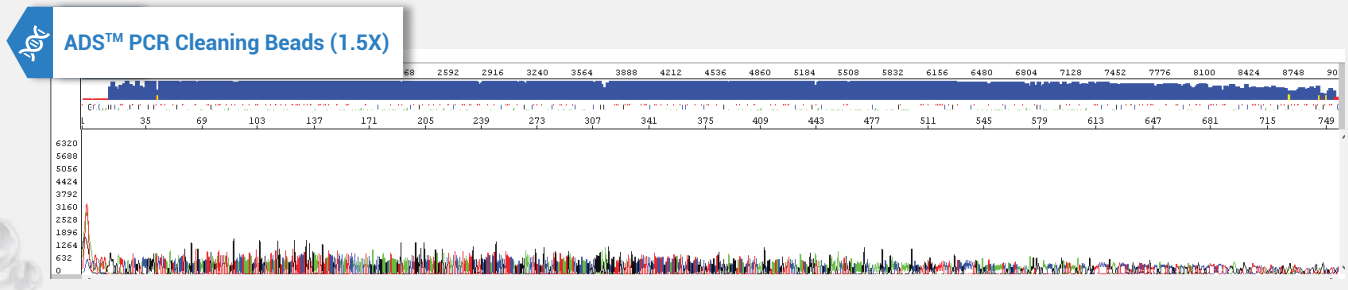


Preparing high-quality sequencing templates is one of the key parameters for sequencing success. Among the various sequencing template types, PCR templates, along with plasmid templates, are the most popular and need careful preparation for optimal sequencing quality.

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.

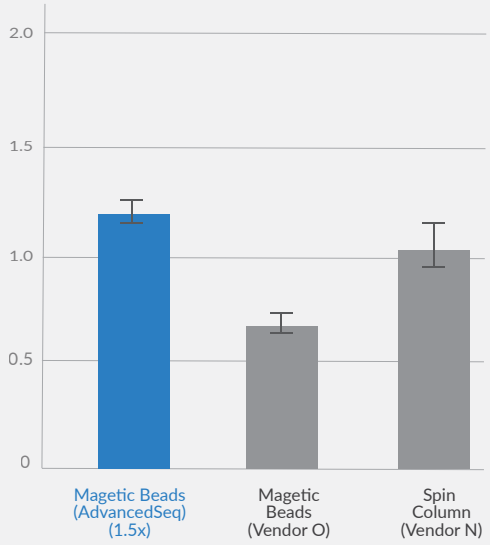
# ADS™ PCR Cleaning Beads

## 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.

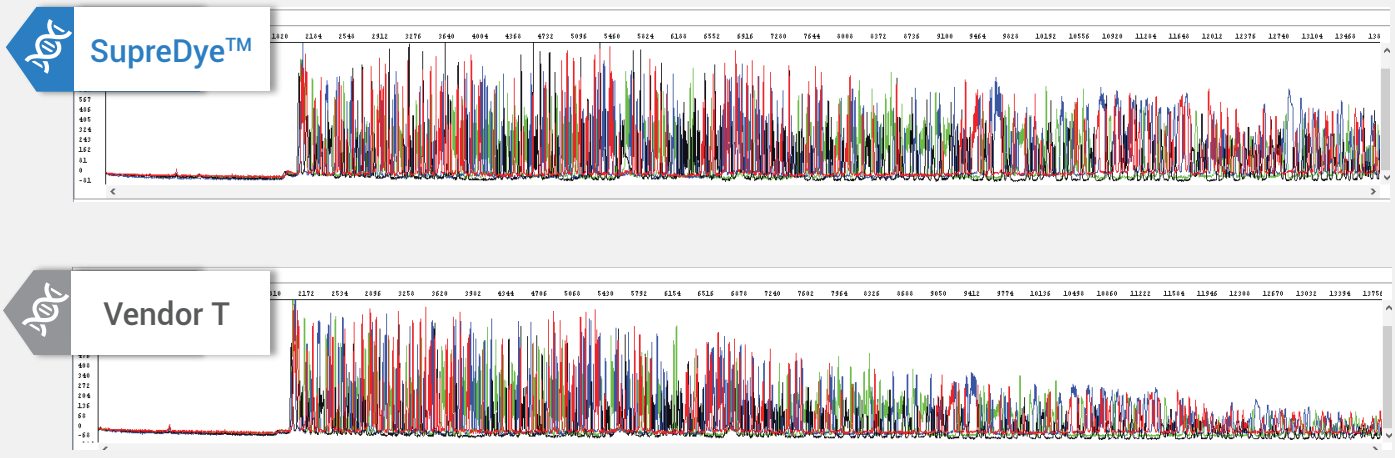
## ORDERING INFORMATION

| 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).

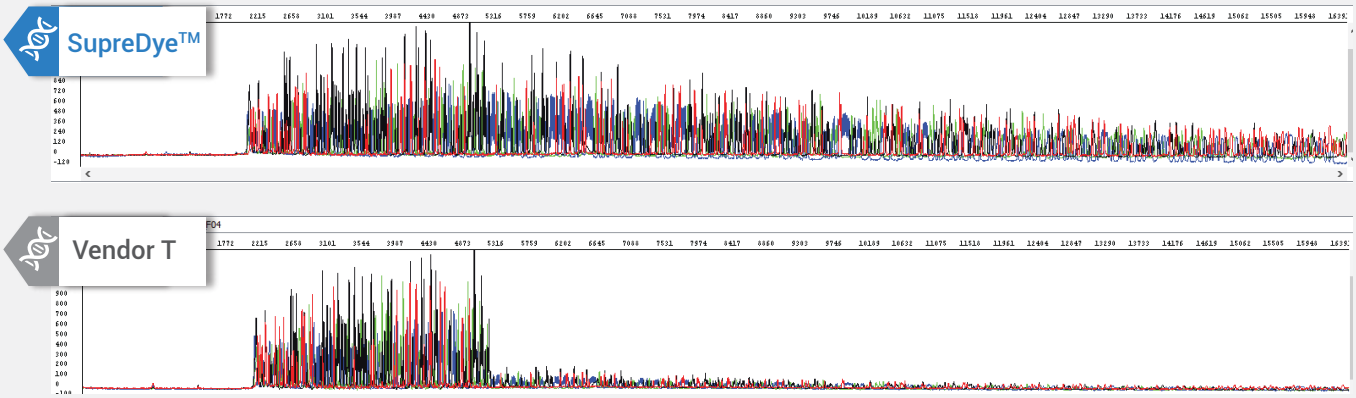
# SupreDye™ Cycle Sequencing Kits

## 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 |

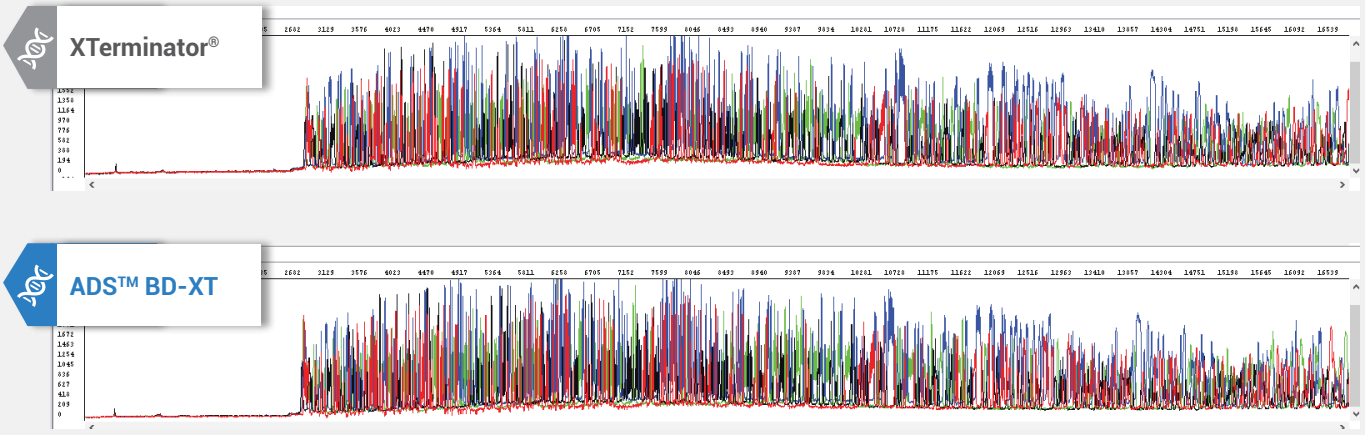
| Product Name | Unit Size | Catalog # |
|---|---|---|
| SupreDye™ dGTP BD1 Cycle Sequencing Kit | 100 rxn | 061002 |
| | 2500 rxn | 061020 |
| SupreDye™ dGTP BD3 Cycle Sequencing Kit | 100 rxn | 065002 |
| | 2500 rxn | 065020 |
| ADS™ 5x Sequencing Buffer | 28 ml | 070028 |
| | 233 ml | 070233 |

# 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

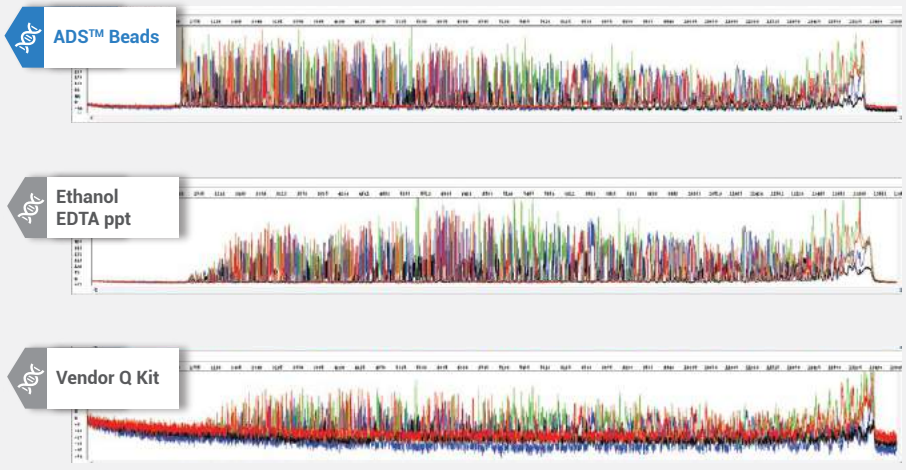
### ORDERING INFORMATION

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

### Choosing the Right Kit for Sequencing Reaction Cleanup

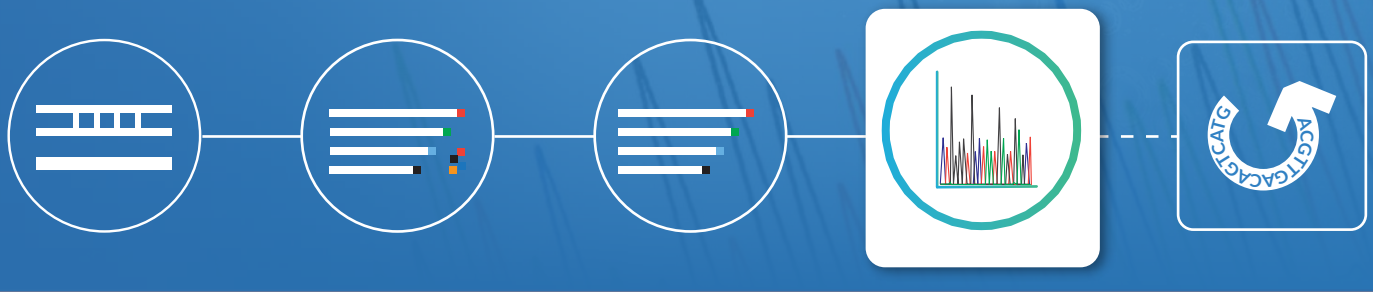
Both kits can effectively remove sequencing contaminants and achieve desired sequencing results. With ADS™ BD-XT Purification Kit, the washing steps, which are part of the protocol for ADS™ Sequencing Reaction Cleaning Beads, can be eliminated. The purified products can then be directly loaded to the 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



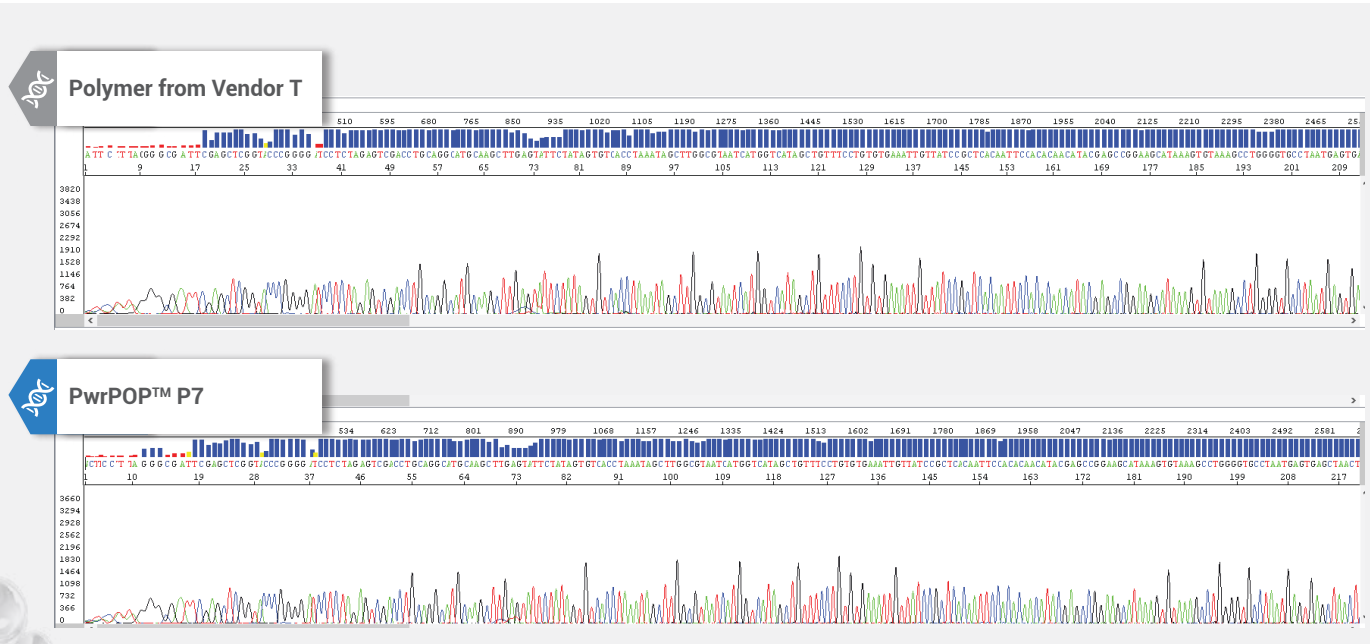

Sequencing raw data comparison for the same sequencing reaction purified by ADS™ Beads (top panel), ethanol purification (middle panel), and Vendor Q kit spin column purification (bottom panel). Both ethanol precipitation and Vendor Q kit lose small extension products and generate significantly reduced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

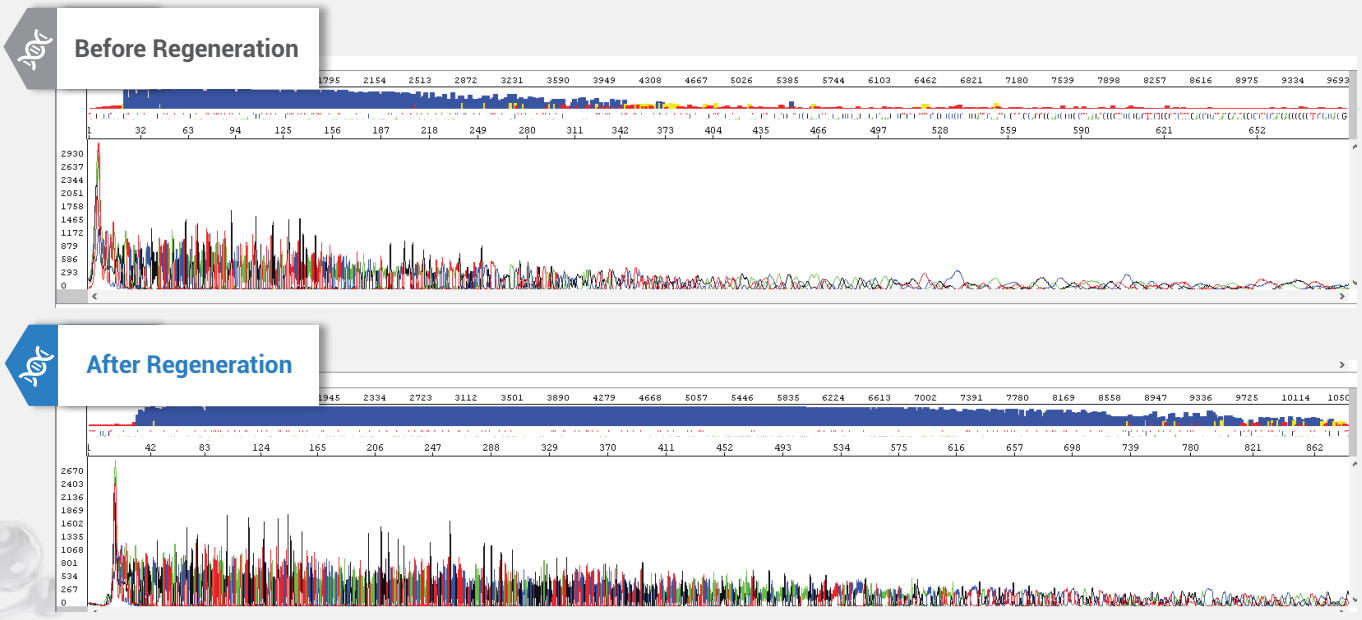
## ORDERING INFORMATION

| 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.

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your first choice for scientific solutions

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