# AxyPrep™ Mag FragmentSelect-I Protocol

(Fragment Size Selection for Illumina Genome Analyzer and Life Technologies SoLiD)

## Introduction

The AxyPrep Mag FragmentSelect-I purification kit utilizes a unique paramagnetic bead technology for quick high-throughput DNA size selection for the Illumina and Life Technologies next generation sequencing platforms . DNA of diverse fragment sizes differentially binds to beads, in response to the AxyPrep FragmentSelect-I concentration. The higher the concentration of AxyPrep Mag FragmentSelect-I, the smaller size DNA fragments bound to beads.

AxyPrep Mag FragmentSelect-I consists of two major stages. The first stage is to determine the optimal AxyPrep Mag FragmentSelect-I ratio to DNA sample ratio for target DNA size selection. The second stage utilizes the determined AxyPrep Mag FragmentSelect-I ratio for isolation of target DNA fragment size.

There are two different ways to isolate target DNA using the FragmentSelect-I kits. If the goal is to remove smaller DNA fragments a one step process is available. However, if the goal is to remove both the larger or smaller DNA fragments a two step process is recommended.

The protocol consists of:

- Binding larger DNA to the paramagnetic beads,
- Binding the target DNA off beads,
- Targeting DNA recovery,
  - Performing ethanol washes to remove impurities
  - A final elution step to recover only the targeted DNA fragment.

DNA fragment sizes larger or smaller than the target size is mostly removed during the binding and washing-off steps. The purified DNA product is essentially free of contaminants. The binding of DNA to magnetic beads is based on the amount of AxyPrep FragmentSelect-I added into DNA solution. The more AxyPrep Mag FragmentSelect-I is added; the smaller size DNA can be isolated. Depending on the amount of the AxyPrep Mag FragmentSelect-I added, DNA fragment ranging from 300 bp – 1000 bp can be isolated from a population of

DNA fragments.

## AxyPrep Mag FragmentSelect-I can be used in the following applications:

- PCR
- Sequencing (Sanger and Next Generation)
- Cloning

## Process Overview

**A- One-step process to remove smaller DNA fragments:**

1. Add AxyPrep Mag FragmentSelect-I to the DNA solution for larger fragments to bind to the magnetic beads (decreasing the amount of AxyPrep Mag FragmentSelect-I produces less precipitation power that allows the binding of larger size DNA). DNA fragments less than the target size remains in solution.
2. Use a magnetic plate, the Axygen IMAG, to separate the smaller DNA (in solution) from the target fragments (bound to the beads).
4. Remove and discard the supernatant.
5. While the magnetic beads are retained on the magnet wash the beads twice with 80% ethanol to remove salt and contaminants.
6. Elute target size DNA from magnetic beads and transfer to a new plate.

**B-Two-step process to isolate larger or smaller DNA fragments:**

1. Add AxyPrep Mag FragmentSelect-I into DNA solution to allow binding of large size DNA onto magnetic beads (this step removes DNA larger than target DNA).
2. Separate beads from clear supernatant.
3. Add additional beads to the supernatant in step 3 to facilitate binding of the target DNA onto the magnetic beads.
4. Wash beads twice with 80% ethanol to remove salt and contaminants.
5. Elute target DNA.

## Specifications

The AxyPrep Mag FragmentSelect-I kit can be performed in a tube and 96-well formats. The following table illustrates the number of reactions an AxyPrep FragmentSelect-I kit can purify depending on the volume of DNA solution

## AxyPrep FragmentSelect-I Kits

| AxyPrep Mag FragmentSelect-I Products | P/N |
|---|---|
| AxyPrep Mag FragmentSelect-I - 5 mL | Mag-FRAG-I-5 |
| AxyPrep Mag FragmentSelect-I - 50 mL | Mag-FRAG-I-50 |
| AxyPrep Mag FragmentSelect-I - 250 mL | Mag-FRAG-I-250 |

| DNA Reaction Volume (96 well, µL) | Mag-FRAG-I-5 (# reactions) | Mag-FRAG-I-50 (# reactions) | Mag-FRAG-I-250 (# reactions) |
|---|---|---|---|
| 100 | 18 | 175 | 875 |
| 50 | 35 | 350 | 1750 |

## Materials supplied in the Kit:

**- AxyPrep Mag FragmentSelect-I paramagnetic bead Solution**

**- Store at 4°C upon arrival (do NOT freeze) for up to 12 months**

**- Mix the reagent well at room temperature** to completely resuspend beads prior to use. The solution should appear homogenous.

## Materials supplied by the User:

### *Consumables & Hardware:*

| Name | Recommended Model | Recommended Vendor and P/N |
|---|---|---|
| 96-well PCR reaction plate | 96-well round/ flat bottom microtiter plate. Plate selection depends on the PCR reaction volume | Corning, Inc., www.corning.com<br># 3797, 96 well round bottom<br># 3591, 96 well flat bottom<br># 3957, 0.5 mL v bottom 96<br># 3365, 360 µl round 96<br># 3364, 360 µl flat 96<br># 3371, 96 clear pro |
| | 96-well cycling plate | Axygen, PCR-96-FS-C, PCR-96M2-HS-C, www.axygen.com |
| 384-well PCR reaction plate | 384 well cycling plate | Axygen, PCR-384M2-C, www.axygen.com |
| PCR Plate Seals | Easy Peel Heat Sealing Foil | Axygen, MF-111, www.axygen.com |
| Liquid handling robotics | Compatible with open platform robotics | Contact Axygen Biosciences Technical support for compatible AxyPrep Mag methods and accessories for automation |
| Multichannel hand pipette | AxyPet | Single, 8 and 12 Multichannel |

## Handheld Magnetic Separation Devices Selection Guide:

The handheld magnetic devices have been optimized for different AxyPrep Mag protocols. These magnets address different volumes for tubes and plate types.

| Protocol | Manufacturer | Part number | Plate description | Plate Material | Part Number |
|---|---|---|---|---|---|
| AxyPrep Mag Kits | Axygen | SCT-050-SS-C | 0.5 ml Self Standing Screw cap tube | Polypropylene | IMAG-12T |
| | Axygen | SCT-150-SS-C | 1.5 ml Self Standing Screw cap tube | Polypropylene | |
| | Axygen | SCT-200-SS-C | 2.0 ml Self Standing Screw cap tube | Polypropylene | |

| Protocol | Manufacturer | Part number | Plate description | Plate Material | Part Number |
|---|---|---|---|---|---|
| AxyPrep Mag PCR Clean -up<br>AxyPrep FragmentSelect-I<br>AxyPrep FragmentSelect-R<br>AxyPrep Mag DyeClean<br>AxyPrep Mag DNA Normalizer<br>AxyPrep Mag Blood gDNA<br>AxyMag FFPE (DNA-RNA- miRNA)<br>AxyPrep Mag Plasmid Kit<br>AxyPrep Mag Tissue gDNA | Corning | 3364 | 96 flat 360 µl | Polypropylene | IMAG-96P |
| | Corning | 3591 | 96 flat bottom | Polystyrene | |
| | Corning | 3365 | 96 round 360 µl | Polypropylene | |
| | Corning | 3371 | 96 clear pro round | Polypropylene | |
| | Corning | 3797 | 96 round bottom | Polystyrene | |
| | Corning | 3957 | 96 v bottom 0.5 mL | Polypropylene | |
| | Axygen | PCR-96-FS-C | 96 PCR full skirt | Polypropylene | |
| | Axygen | PCR-96M2-HS-C | 96 PCR half skirt | | |
| | Corning | 3959 | 96 round bottom 1 ml | | |
| | Corning | 3961 | 96 round bottom 2 ml | | |

***Reagents:***

| Reagents | Application |
|---|---|
| 100% ethanol | Binding reagent |
| 70% ethanol | Washing solvent |
| 10 mM Tris-HCl, pH=8.0 | DNA elution |
| reagent grade water | |
| 10 mM Tris-HCl pH 8.0, 1 mM EDTA | |

## TWO-STEP PROCEDURE WITH DNA AXYPREP FRAGMENTSELECT- I
## TARGET FRAGMENT SIZE: 500bp

### Procedure For 96-well Plate:

1. **Add 65 µl of room temperature AxyPrep FragmentSelect-I to 50 µl of the DNA solution (DNA target size is 500 bp). Then Pipette-mix five times to obtain a homogenous solution and incubate at room temperature for 5 minutes. During this time the fragment sizes greater than 500bp will bind to the beads, while smaller DNA fragments will remain in solution.**

**NOTE:** To obtain a fragment size of 500 bp the ratio of AxyPrep FragmentSelect-I to DNA solution is 1.3X. If a DNA volume other than 50 µl is used, adjust the AxyPrep FragmentSelect-I volume accordingly to maintain the ratio.

**NOTE:** Prior to adding the AxyPrep FragmentSelect-I to the DNA solution briefly vortex the paramagnetic beads.

2. **After the five minute incubation in step 1, place the plate on an Axygen IMAG to separate the paramagnetic beads from the solution containing the target DNA. Incubate at room temperature for 1 minute or until solution clears.**
3. **Transfer the supernatant to a fresh plate.**
4. **Add 10ul of FragmentSelct-I to the supernatant from step 3 and gently pipette-mix five times. Incubate at room temperature for 5 minutes**
5. **Place the 96-well plate on the Axygen IMAG for 2 minutes to allow for separation or until the solution is clear.**

**NOTE:** Target DNA is bound to beads.

6. **Remove the cleared supernatant and discard.**
7. **While the plate is on the magnet, wash the beads with 200 µl of 80% ethanol by carefully pipetting up/down five times. Discard the ethanol after the wash.**

**NOTE:** While washing the paramagnetic beads pipette the ethanol on the opposite side of the beads so as NOT to disturb the beads.

8. **Repeat Step 6 and 7.**
9. **Remove 80% ethanol after the final wash and let the beads dry for 5 minutes at room temperature.**

**NOTE:** A longer drying time may be necessary to remove any residual ethanol that may

interfere with downstream applications. However, be careful not to over dry the beads (e.g. placing the beads at 37 °C) because this will inhibit DNA recovery.

10. **Remove the plate from the magnet and resuspend beads in 20 µl of molecular biograde water to elute the target DNA.**
11. **Place the plate on the magnet to separate the beads from solution and incubate for 1 minute or until solution becomes clear.**
12. **Transfer the clear DNA solution to a fresh plate.**
13. **Examine the DNA size distribution and concentration with Bioanalyzer.**

**NOTE:** ***To recover DNA fragments smaller than 500bp such as 300bp, use 1.6X for the initial bind.***

## ONE-STEP SIZE SELECTION PROCEDURE WITH DNA AXYPREP FRAGMENTSELECT- I

1. **Add 70 µl of room temperature AxyPrep FragmentSelect-I to 50 µl of the DNA solution (DNA target size is > 500 bp). Then Pipette-mix five times to obtain a homogenous solution and incubate at room temperature for 5 minutes. During this time the fragment sizes larger than 500 bp will bind to the beads, while DNA fragments 500 bp and smaller will remain in solution.**

**NOTE:** To obtain a fragment size of 500 bp the ratio of AxyPrep FragmentSelect-I to DNA solution is 1.4X. If a DNA volume other than 50 µl is used, adjust the AxyPrep FragmentSelect-I volume accordingly to maintain the ratio.

**NOTE:** Prior to adding the AxyPrep FragmentSelect-I to the DNA solution briefly vortex the paramagnetic beads.

**2. After the incubation in step 1, place the plate on the Axygen IMAG for 2 minutes or until solution clears to separate the paramagnetic beads from the solution.**

**3. Remove the cleared supernatant and discard.**
**4. While the plate is on the magnet wash the beads with 200 µl of 80% ethanol by pipetting ethanol five times.**

**NOTE:** While washing the paramagnetic beads pipette the ethanol on the opposite side of the beads so as NOT to disturb the beads.

**5. Repeat Steps 3 – 4 once.**
**6. Completely remove 80% ethanol after the final wash and let the beads dry for 5**

**minutes at room temperature.**

**NOTE:** A longer drying time may be necessary to remove any residual ethanol that may interfere with downstream applications. However, be careful not to over dry the beads (e.g. placing the beads at 37 °C) because this will inhibit DNA recovery.

**7. Remove the plate from the magnet and resuspend beads in 20 µl of molecular biograde water to elute the target DNA.**

**8. Place the plate on the magnet and incubate for 1 minute or until solution becomes clear to separate the beads from solution.**

**9. Transfer the clear DNA solution to a fresh plate.**

**10. Examine the DNA size distribution and concentration with Bioanalyzer.**

*Please contact Axygen Biosciences for sales support at: axgsales@corning.com and for technical support at: axgsupport@corning.com*

** The PCR process is covered by patents owned by Roche Molecular Systems, Inc., and F. Hoffman-La Roche, Ltd.*