



Fusion BioLabs DNA Aptamer Library Kit Manual

(Magnetic Beads-Based -SELEX Module)

SKU# DAL-01(N62) or DAL-01(N40): DNA Aptamer Library Kit

Product Overview

Fusion BioLabs offers a validated DNA Aptamer Library Kit (DAL-01) to streamline your custom DNA aptamer development workflows. This kit provides sufficient ssDNA library oligos and optimized primers for primary library construction during Round 1 SELEX, as well as secondary library construction for 2 to 4 independent aptamer selection projects (depending on your specific SELEX strategy). With customizable options and robust performance, our kit bypasses time-consuming library optimization and accelerates your aptamer discovery pipeline.

Key Features

- **High Efficiency:** Pre-validated library and primer sets engineered for specific, high-yield amplification in both Capture SELEX and traditional magnetic bead-based SELEX platforms.
- **Flexibility & Versatility:** Fully compatible with multiple single-stranded DNA (ssDNA) generation protocols:
 1. *Enzymatic Digestion:* Upgraded SELEX workflow using 5'-phosphorylated primers and Lambda Exonuclease digestion.
 2. *Alkaline Denaturation:* Standard strand separation using streptavidin-coated magnetic beads and biotinylated primers.
- **Real-Time Tracking:** Includes 5'-FAM labeled forward primers for binding affinity monitoring, fluorometric quantification, and downstream SELEX tracking.

Kit contents

The following components are included in the Kit.

Vial	Component Description	Concentration	Volume
Vial 1	ssDNA Library Oligos (N62 or N40)	100 μ M	30 μ l
Vial 2	Unmodified Forward Primer	100 μ M	30 μ l
Vial 3	5'-FAM Labeled Forward Primer	100 μ M	30 μ l
Vial 4	Unmodified Reverse Primer	100 μ M	30 μ l
Vial 5	5'-Phosphorylated Reverse Primer	100 μ M	30 μ l
Vial 6	5'-Biotinylated Reverse Primer	100 μ M	30 μ l



All reagents are supplied liquid and ready-to-use. Store at -20°C (reagents are stable for 12 months when properly stored).

ssDNA Library Pool Configurations

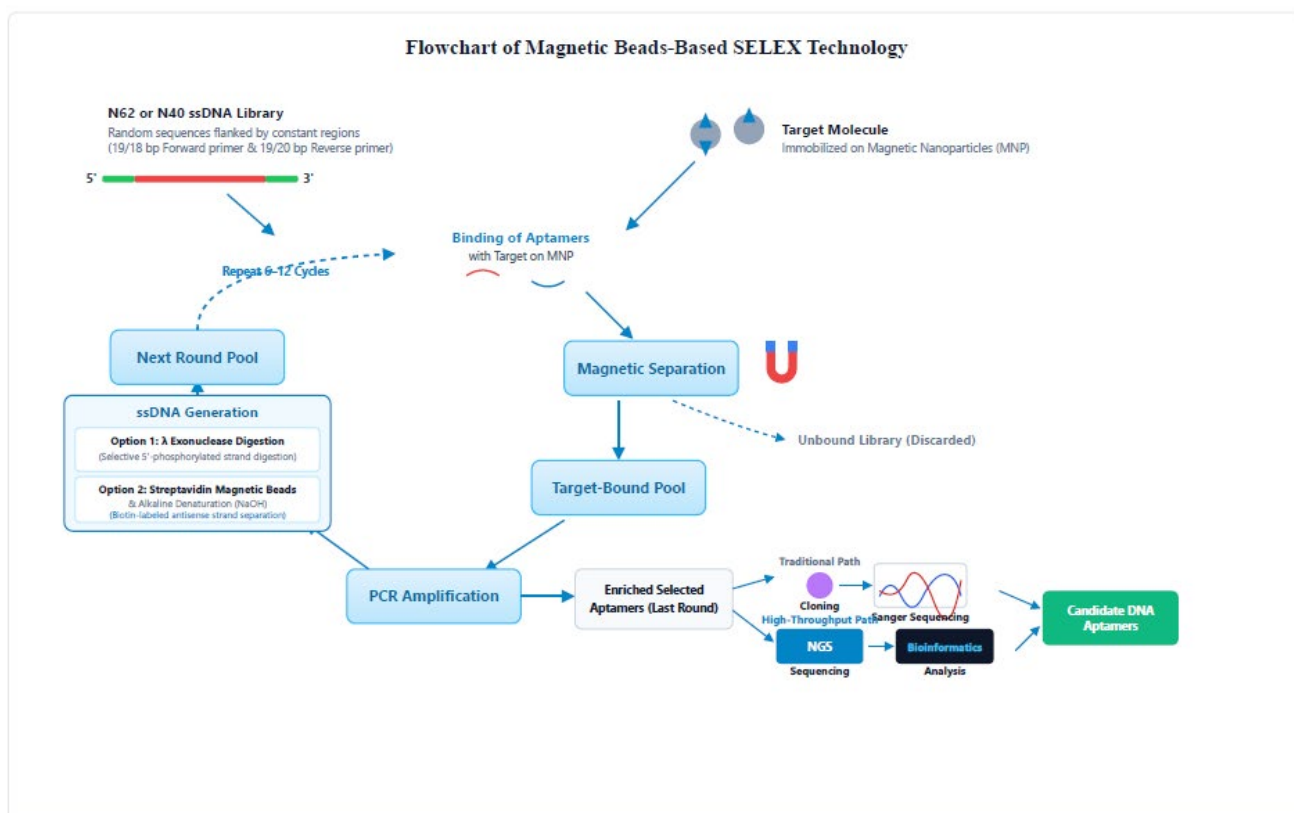
The DAL-01 kit is supplied with your choice of random region configuration:

SKU Option	Forward Primer Region	Random Region	Reverse Primer Region	Total Length
DAL-01 (N62)	19 bp	N62 (62 nt)	19 bp	100 nt
DAL-01 (N40)	18 bp	N40 (40 nt)	20 bp	78 nt

Kit Usage

- Primary library construction for 1st round SELEX.
- Secondary library construction for the iterative SELEX.

Flowchart of magnetic beads based-SELEX technology to find the candidate DNA aptamer.



Protocols



In this protocol, the magnetic beads based-SELEX technology was used to select the aptamer bound to your target *in vitro*. The entire selection process includes 10-12 rounds, which contains steps listed as follows.

1. Target Protein Conjugation on Magnetic Nanoparticles (MNP)

- 1) Weigh out 3.0 mg conjugate magnetic nanoparticles (MNP) (e.g., Dynabeads™ M-270 Epoxy).
- 2) Wash the beads 3 times with 1 mL of 0.1 M sodium phosphate buffer (pH 7.4).
- 3) Resuspend washed beads in phosphate buffer, add target protein/peptide, and add 3 M $(\text{NH}_4)_2\text{SO}_4$ stock solution.
- 4) Incubate for 16–24 h at 37°C with slow tilt rotation.
- 5) Place tube on magnetic rack for 2 min, discard supernatant, and wash coated beads 4 times with PBS (pH 7.4) containing 0.1% BSA or 0.5% Tween-20.
- 6) Resuspend coated beads to 6.0 $\mu\text{g}/\mu\text{L}$ in PBS with 0.1% BSA. (*Prepare uncoated MNP beads in parallel for counter-selection*).

*Prepare uncoated MNP beads without a target in parallel for counter-selection.

2. SELEX for the first round

- 1) Dilute up to 30 μL (1–3 nmol for Round 1) of Vial 1 ssDNA library into 450 μL PBS-T (PBS + 0.5% Tween-20).
- 2) Denature at 95°C for 5 minutes, then snap-cool on ice for 15 minutes.
- 3) Add 50 μL target-coated MNP beads and 50 μL BSA (1 mg/).
- 4) Incubate at room temperature with end-over-end (EOE) rotation for 30–60 minutes.
- 5) Separate MNP beads using a magnetic rack and discard unbound library.
- 6) Wash beads 10–15 times with 1 mL PBS-T.
- 7) Resuspend washed beads in 50 μL PCR-grade water.

3. Enriched Pool Amplification and dsDNA Purification

- 1) PCR Setup

Reaction Component	Volume	Thermal Cycling Parameters	
2 x PCR Master Mix	200 μL	Initial denaturation	94°C for 2 min
Enriched ssDNA Pool	50 μL	15-20 PCR cycles	Denature 94°C for 30 sec
Forward Primer (10 μM)*	8 μL		Anneal 51°C for 30 sec
Reverse Primer (10 μM)*	8 μL		Extend 72°C for 90 sec
Nuclease-Free Water	134 μL	Final Extension 72°C for 10 min	
Total Volume	400 μL	Hold 4°C	



Primer Selection Guide:*	Note: Recommended for our PCR condition. Optimization maybe needed.
○ Alkaline Denaturation Workflow: Vial 2 (Forward) + Vial 6 (Biotinylated Reverse). ○ Lambda Exonuclease Workflow: Vial 2 (Forward) + Vial 5 (Phosphorylated Reverse). ○ Fluorometric Tracking / Real-time Monitoring: Use Vial 3 (FAM-Labeled Forward).	

2) dsDNA purification

Purify double-stranded DNA using standard ethanol precipitation or any commercial PCR & DNA Cleanup Kit. An ethanol precipitation method is followed: add 1/10th volume of 3 M sodium acetate (pH 5.2) and 2.5 volumes of ice-cold absolute ethanol to the DNA sample, mix, and incubate at -20°C for at least one hour or overnight. Centrifuge the sample to pellet the DNA, and then wash the pellet with 70-80% ethanol to remove salts, and dry the pellet before re-dissolving it.

4. ssDNA Generation for Subsequent Selection Cycles

1) Streptavidin Bead Option:

- 1) Take 200 µl for first round and 100 µl for the continual rounds of Dynabeads M-280 streptavidin-coated magnetic beads suspension.
- 2) Wash three times with a 1× B & W buffer (5 mM Tris-HCl, 0.5 mM EDTA, 1 M NaCl, 20 mM KCl, 5 mM MgCl₂, 0.1% BSA, 0.05% Tween-20. Adjust pH to 7.5.).
- 3) Incubate the biotinylated PCR products with pre-washed beads in an EOE mixer for 30 min at room temperature.
- 4) Add 50 µl of a freshly prepared 150 mM NaOH solution and incubate at room temperature for 5 min.
- 5) Neutralize the eluted ssDNA with 50 µl HCl (150 mM).
- 6) Dilute up to 100 µl of the above enriched ssDNA library in final 450 µl PBS-T.
- 7) Denature at 95°C for 5 minutes and then left on ice to cool for 15 minutes.
- 8) Add 50 µl of uncoated MNP beads to the ssDNA tube and 50 µl 1 mg/ml BSA (molecular grade), and vortex gently.
- 9) Incubate the mixture at room temperature with an EOE (ender-over-ender) mixer for 30-60 minutes.
- 10) Separate MNP beads with magnetic rack.
- 11) Collect the supernatant for the next round of positive SELEX following the procedure listed on **Step 3 of 2. SELEX for the first round.**

2) Lambda Exonuclease Option:

- 1) Set-up the reaction as follows:



Components	500 µl REACTION
PCR reaction from Step 3	Up to 400 µl
Lambda Exonuclease Reaction Buffer (10X)	50 µl (1X)
Lambda Exonuclease	2 µl (10 units)
Nuclease-Free H ₂ O	up to 500 µl

- 2) Incubate at 37°C for 30 minutes.
- 3) Stop reaction by adding EDTA to 10 mM.
- 4) Heat Inactivation 75°C for 10 minutes.
- 5) To clean up treated samples, we recommend using one of the following steps:
 - a. Column clean up (many supplier provided), or
 - b. Running the reaction on an agarose gel, and then extracting the DNA, or
 - c. Performing a phenol/chloroform extraction followed by ethanol precipitation.

5. Monitoring the enrichment progress of SELEX

- Generally 12-16 SELEX rounds is enough to get high specificity and affinity DNA aptamer candidates.
- Use 4 µl of the fraction you collected in counter-elution and target-binding elution.
- Measure the amount of FAM labelled ssDNA pool in each round sample according to the **Supplementary protocol 1**.
- Calculate the ratio of the amount of ssDNA pool eluted in the target binding step compared to the background elution step.
- The expected results: ratio should be increased from ~1 (round 1) up to 5-20. If reached the platform. SELEX process could be terminated.

6. Cloning and sequencing the candidate DNA aptamers

- After the SELEX was completed, do the final PCR amplification according to the Step of PCR setup and PCR protocol, but using the unmodified forward primer and reverse primer pair from Vial 2 and Vial 4.
- Purify the PCR amplification products (final dsDNA pool) using your favorite methods.
- For Sanger sequencing: cloning purified PCR fragments into your TA cloning compatible sequencing vector. We generally pick up 96 clones to identify the unique DNA aptamer candidates.
- For high-throughput sequencing (HTS) or next-generation sequencing (NGS): send your purified PCR fragments to your service provider. We recommend using Illumina Miseq platform.

7. Affinity characteristics of aptamer candidates

Aptamer candidates with high enrichment, low free energy level and the large difference in the secondary structure will be selected for binding assay. All experiments should be carried out under dark conditions.

- Synthesize the aptamer candidates with 5'-end labelled with FAM.



- Add individual aptamer candidate with varying concentration (e.g. 10-500 nM) to a specific amount of target-coated MNP beads or uncoated MNP beads according to the strategy of the SELEX procedure.
- Incubate the mixture at room temperature for 30 min under EOE mild rotation.
- Separate beads with magnetic rack and collect the supernatant to measure the amount of 5'-FAM-labelled candidate individually.
- The nonlinear fitting formula will be employed to determine the K_D of an aptamer candidate to its target. We recommend using GraphPad Prism Software to simplify the calculation.

Supplement protocol 1

How to measure the amount of FAM labelled single stranded oligos

You can measure the concentration of FAM-labeled single-strand oligonucleotides (ssDNA) using a combination of UV absorbance and fluorescence spectroscopy. Because both the nucleic acid and the FAM dye absorb light, it is important to correct for the contribution of the dye to get an accurate measurement of the oligo concentration.

Method 1: Using a NanoDrop with the MicroArray module

For fluorescently labeled oligos, the MicroArray module in NanoDrop software can automatically correct for the dye's absorbance.

1. **Select the "MicroArray" module** and the "Oligo" option for your sample type.
2. **Measure the absorbance** of your sample. The instrument will measure both the absorbance of the FAM dye (at its maximum wavelength, λ_{max} , around 494 nm) and the absorbance of the nucleic acid (at 260 nm).
3. **The software will correct the A260 reading** using a correction factor and an oligo-specific extinction coefficient, providing a more accurate concentration value for your labeled oligo.

Method 2: Calculating concentration manually

If you do not have software with an automatic correction feature, you can calculate the concentration manually using the Beer-Lambert Law, factoring in the dye's contribution to the A260 reading. This requires knowing the extinction coefficients for both the FAM dye and the unlabeled oligo.

Steps for manual calculation

1. **Measure absorbance:** Use a spectrophotometer to measure the absorbance of your sample at two wavelengths:
 - **A₂₆₀:** To measure the concentration of the oligo bases.
 - **A₄₉₄:** To measure the concentration of the FAM dye.
2. **Look up or calculate extinction coefficients:**



- $\epsilon^{\text{oligo}/260}$: The extinction coefficient of your specific, unlabeled oligo at 260 nm. This can be calculated using online tools like IDT's OligoAnalyzer.
 - $\epsilon^{\text{FAM}/260}$: The extinction coefficient of FAM at 260 nm (approx. $9,000 \text{ M}^{-1}\text{cm}^{-1}$).
 - $\epsilon^{\text{FAM}/494}$: The extinction coefficient of FAM at its maximum absorption wavelength (494 nm, approx. $75,000 \text{ M}^{-1}\text{cm}^{-1}$).
3. **Calculate dye concentration:** Using the A_{494} reading, calculate the molar concentration of the FAM dye:
- $C_{\text{FAM}} = A_{494} / \epsilon^{\text{FAM}/494}$
4. **Calculate oligo concentration:** Use the FAM concentration to subtract the dye's contribution from the total A_{260} reading, then solve for the oligo concentration:
- $A^{\text{total}/260} = A^{\text{oligo}/260} + A^{\text{FAM}/260}$
 - $A^{\text{oligo}/260} = A^{\text{total}/260} - (C_{\text{FAM}} \cdot \epsilon^{\text{FAM}/260})$
 - $C_{\text{oligo}} = A^{\text{oligo}/260} / \epsilon^{\text{oligo}/260}$

Method 3: Using a fluorometer

For greater sensitivity, especially with low-concentration samples, you can use a fluorometer with a standard curve.

1. **Prepare standards:** Create a series of dilutions of a stock FAM-labeled oligo with a known concentration.
2. **Generate a standard curve:** Measure the fluorescence of the standards at FAM's excitation (**494 nm**) and emission (**520 nm**) wavelengths.
3. **Measure your sample:** Measure the fluorescence of your unknown sample.
4. **Determine concentration:** Compare the sample's fluorescence to the standard curve to determine its concentration.

Tips for accurate measurement

- **Remove excess dye:** If your oligo was labeled in-house, ensure you have removed any free dye that may remain from the labeling reaction. Free dye can significantly alter your readings.
- **Use calibrated equipment:** Regardless of the method, ensure that all equipment especially pipettes, are properly calibrated to avoid measurement errors.
- **Mix thoroughly:** Before taking any measurement, ensure that your sample is completely mixed and homogeneous.
- **Run controls:** For fluorescence methods, include blank controls to account for any background fluorescence from the buffer or plate.