Fusion BioLabs DNA Aptamer Development Kit (Capture-SELEX module)

SKU# DAL-02: DNA Aptamer Development Kit (Capture-SELEX module)

Product Overview

Fusion BioLabs offers a fully validated DNA Aptamer Development Kit (Capture-SELEX module) for your own DNA aptamer development. This kit is a fully validated DNA aptamer development kit, includes all of the reagents up to SELEX procedures using Fusion BioLabs developed capture-SELEX platform.

- ssDNA library oligos and primers for your primary library construction and secondary library construction for the iterative SELEX up to 15 rounds.
- Target-bound and unbound ssDNA aptamer pool partition using streptavidin magnetic beads.
- Counter and/or negative bound ssDNA aptamer pool removal using streptavidin magnetic beads.
- PCR master mix for dsDNA amplification and purification.
- ssDNA aptamer pool preparation using lambda exonuclease digestion.
- ssDNA aptamer pool purification using quick and simple column-based technology.

The DNA Aptamer Development Kit (Capture-SELEX module) is fully optimized for one Capture-SELEX project (enough for 15 rounds of SELEX) to get your DNA aptamer candidates within 4 weeks.

Kit contents
The following components are included in the Kit.

Component	Quantity
Vial 1: ssDNA Library Oligos	20 µl, 100 µM
Vial 2: 5'-Biotinylated Capture Oligos	60 µl, 100 µM
Vial 3: Forward Primer	20 µl, 100 µM
Vial 4: 5'-FAM Labeled Forward Primer	20 µl, 100 µM
Vial 5: Reverse Primer	20 µl, 100 µM

Vial 6: 5'-Phosphorylated Reverse Primer	20 µl, 100 µM
Vial 7: Streptavidin-Coated Magnetic Beads	3.0 ml
Vial 8: Lambda Exonuclease Reaction Buffer (10X)	100 µl
Vial 9: Lambda Exonuclease (5 units/µl)	20 µl
Vial 10: ssDNA Binding Buffer	15 ml
Vial 11: ssDNA Preparation Buffer	15 ml
Vial 12: ssDNA Wash Buffer	9.0 ml
Vial 13: PCR-grade Water	1.5 ml
Vial 14: 2x PCR Master Mix	3.0 ml
ssDNA Cleanup Column I	30 columns
ssDNA Cleanup Column II	30 columns
Collection Tubes	60 tubes
Nuclease-free Tubes	30 tubes

- Store at -20°; reagents are guaranteed stable for 12 months when properly stored.

Kit Usage

- Primary ssDNA library construction for 1st round SELEX.
- Secondary ssDNA library construction for the iterative SELEX.
- A fully validated development kit utilizing capture-SELEX platform is available for both small and large molecular targets.

Protocols

Materials needed but not included in the kit

- 5x Capture-SELEX Buffer (**5x CSB**): 200 mM HEPES, 1.25 M KCl, 100 mM NaCl, 25 mM MgCl2. Adjust pH to 7.4.
- 1x Capture-SELEX Buffer (**1x CSB**) with Tween-20 (**1x CSB+Tween**): Diluted 5x CSB and add 0.002% Tween-20.
- **Target Solution**: 1 mM target-compound in 1xCSB without Tween-20.
- Binding and Wash Buffer (**B&W buffer**): 5 mM Tris-HCl, 0.5 mM EDTA, 1 M NaCl, 0.01% Tween-20. Adjust pH to 7.5.

- PCR Purification Kit or DNA ethanol precipitation reagents

In this protocol, the Capture-SELEX technology was used to select the aptamer bound to your target *in vitro*. The entire selection process includes 12-16 rounds, which contains steps listed as follows.

1. **Hybridization of ssDNA pool and capture oligos**
    - Mix 10 µl **5x CSB** (first round 40 µl), 20 µl ssDNA pool (first round 20 µl, Vial 1), 2 µl biotinylated capture oligos (Vial 2, first round 30 µl), add 18.5 µl of PCR water (first round 110 µl) in 2.0 ml centrifuge tube
    - Denature at 95°C for 5 min, then gradually cool down and incubate at room temperature for 60 min in an end-over-end (EOE) mixer.

2. **Streptavidin-coated magnetic beads preparation**
    - Take 125 µl of magnetic beads suspension (first round 1.0 ml).
    - Wash three times with 800 µl **B& W buffer**.
    - Remove supernatant with magnetic rack and suspend beads in 150 µl **1x CSB + Tween** (first round 300 µl).

3. **ssDNA pool immobilization**
    - Add the 50 µl (first round 200 µl) from the previous 1.1 hybridization reaction to the suspend beads.
    - Leave in an EOE mixer at room temperature for 60 min or overnight with mild shaking.
    - Separate beads with magnetic rack.

4. **Weak binders removal and enriched pool elution**
    - Suspend beads in 500 µl 1x CSB+Tween, EOE rotor for 5 min at room temperature, separate beads with magnetic rack.
    - Suspend beads in 500 µl 1x CSB+Tween, heat at 30°C for 2 min, vortex slightly, heat 30°C for 2 min, vortex slight, heat 30°C for 2 min, separate beads with magnetic rack.
    - Counter elution of non-specific binders: prepare an optimal concentration (for example 1mM) of as compound similar to your target molecule in 1x CSB buffer. Suspend beads in 200 µl of the solution, EOE rotor for 30 min at room temperature, separate beads with magnetic rack. Collect supernatant for later SELEX enrichment progress measurement.

- Enriched pool elution of specific binders: prepare an optimal concentration (for example 1mM for the first round, then decrease the concentration later rounds) of your target molecule in 1x CSB buffer. Suspend beads in 200 µl of the solution, EOE rotor for 30 min at room temperature, separate beads with magnetic rack and collect the supernatant for enriched pool amplification of later round SELEX and enrichment progress measurement.

5. Enriched pool amplification

- Column purification of enriched ssDNA pool
  - Add 2 volumes **ssDNA Binding Buffer** to the eluted enriched ssDNA pool and mix.
  - Transfer the sample to the **ssDNA Cleanup Column I** in a Collection Tube and centrifuge at 10,000-16,000 g for 30 seconds. Save the flow-through.
  - Add an equal volume of molecular-grade ethanol (95-100%) to the flow-through, and mix.
  - Transfer the mixture to the **ssDNA Cleanup Column II** in a Collection Tube and centrifuge at 10,000-16,000 g for 30 seconds. Discard the flow-through.
  - Add 400 µl **ssDNA Preparation Buffer** to the column, and centrifuge at 10,000-16,000 g for 30 seconds. Discard the flow-through.
  - Add 700 µl **ssDNA Wash Buffer** to the column, and centrifuge at 10,000-16,000 g for 30 seconds. Discard the flow-through.
  - Add 400 µl **ssDNA Wash Buffer** to the column, and centrifuge at 10,000-16,000 g for 1 min, discard the flow-through. Recentrifuge at 10,000-16,000 g for 1 min, and transfer the **ssDNA Cleanup Column II** into a nuclease-free tube.
  - Add 25 µl **PCR-grade Water** directly to the column matrix and centrifuge at 10,000-16,000 g for 1 min. Collect the eluted and purified ssDNA pool.

- PCR setup and PCR protocol

Component	Amount	PCR Protocol		
2xPCR Master Mix	200 µl	Initial denaturation		94°C for 2 min
Enriched ssDNA pool*	20 µl	20 PCR cycles	Denature	94°C for 30 sec
5'-FAM Labeled Forward Primer (10 µM)	8 µl		Anneal	51°C for 30 sec
5'-Phosphorylated Reverse Primer (10 µM)	8 µl		Extend	72°C for 90 sec
Water, nuclease-free	170 µl	Final Extension		72°C for 10 min
*Comments: add all enriched ssDNA pool from		Hold		4°C, indefinitely

above (~ 20ul).	Note: Recommended for our PCR condition. Optimization maybe needed.

- Aliquot into 8 PCR tubes (50 µl/tube) and set the PCR protocol for amplification
- Combine all 9 tubes into 2 ml centrifuge. This is the total amplified dsDNA (5' phosphorylated dsDNA) pool for make ssDNA for next round SELEX,
- Purified the dsDNA pool using your favorite methods such as a) ethanol precipitation or b) column purification from many lab suppliers.
- Dissolve and/or elute the purified dsDNA pool in 44 µl PCR-grade water.

6. Preparation of ssDNA enriched library for the next round SELEX

- Set-up the reaction as follows:

Components	
5'-phosphorylated dsDNA	44 µl
Lambda Exonuclease Reaction Buffer (10X)	5 µl
Lambda Exonuclease (5 units/µl)	1 µl

- Incubate at 37°C for 30 minutes.
- Stop reaction by adding 1 µl 500 mM EDTA.
- Heat Inactivation 75°C for 10 minutes.
- To clean up treated samples using the above.
    - Add **100 µl ssDNA Binding Buffer** to the lambda exonuclease reaction tube and mix.
    - Transfer the sample to the **ssDNA Cleanup Column I** in a Collection Tube and centrifuge at 10,000-16,000 g for 30 seconds. Save the flow-through.
    - Add an equal volume of molecular-grade ethanol (95-100%) to the flow-through, and mix.
    - Transfer the mixture to the **ssDNA Cleanup Column II** in a Collection Tube and centrifuge at 10,000-16,000 g for 30 seconds. Discard the flow-through.
    - Add 400 µl **ssDNA Preparation Buffer** to the column, and centrifuge at 10,000-16,000 g for 30 seconds. Discard the flow-through.
    - Add 700 µl **ssDNA Wash Buffer** to the column, and centrifuge at 10,000-16,000 g for 30 seconds. Discard the flow-through.
    - Add 400 µl **ssDNA Wash Buffer** to the column, and centrifuge at 10,000-16,000 g for 1 min, discard the flow-through. Recentrifuge at 10,000-16,000

g for 1 min, and transfer the **ssDNA Cleanup Column II** into a nuclease-free tube.

- Add 25 µl **PCR-grade Water** directly to the column matrix and centrifuge at 10,000-16,000 g for 1 min. Collect the eluted and purified ssDNA pool.
- Take 20 µl for the next round SELEX. Keep the leftover in case failed for the next round SELEX.

7. Monitoring the enrichment progress of SELEX

- 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 5-10. If reached the platform. SELEX process could be terminated.

8. Cloning and sequencing the candidate DNA aptamers

- After the Capture-SELEX was completed, do the final PCR amplification according to the Step 5., but using the unmodified forward primer and reverse primer pair from Vial 3 and Vial 5.
- Purify the PCR amplification products (final ssDNA 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.

9. 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.

- Immobilize individual aptamer candidate with varying concentration (e.g. 10-500 nM) via capture oligo to the surface of streptavidin-coated magnetic beads according to the strategy of the SELEX procedure.
- Add constant amount of target or counter selection control (e.g.10 μg/ml) to the above immobilized aptamer candidate solution.
- 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 simply 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:
   - A_{260}: To measure the concentration of the oligo bases.
   - A_{494}: To measure the concentration of the FAM dye.
2. **Look up or calculate extinction coefficients:**
   - $\epsilon^{oligo/260}$**:** The extinction coefficient of your specific, unlabeled oligo at 260 nm. This can be calculated using online tools like IDT's OligoAnalyzer.
   - $\epsilon^{FAM/260}$**:** The extinction coefficient of FAM at 260 nm (approx. 9,000 $M^{-1}cm^{-1}$).
   - $\epsilon^{FAM/494}$**:** The extinction coefficient of FAM at its maximum absorption wavelength (494 nm, approx. 75,000 $M^{-1}cm^{-1}$).
3. **Calculate dye concentration:** Using the A_{494} reading, calculate the molar concentration of the FAM dye:
   - $C_{FAM} = A_{494} / \epsilon^{FAM/494}$
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:
   - $A^{total/260} = A^{oligo/260} + A^{FAM/260}$
   - $A^{oligo/260} = A^{total/260} - (C_{FAM} . \epsilon^{FAM/260})$
   - $C_{oligo} = A^{oligo/260} / \epsilon^{oligo/260}$

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.