

# Best Practices: SulfoLink® Immobilization Kit

Base Pair Biotechnologies provides custom aptamer development services and catalog aptamers to academic, commercial, and government researchers for a variety of applications. To support their efforts we provide this series of aptamer best practices as an introduction to their use.

Additional assistance is provided as needed.



*Tertiary structure of a DNA aptamer  
developed by Base Pair Biotechnologies*

## Introduction

This protocol has been modified for use with the SulfoLink® Immobilization Kit (Cat #44999) for covalent immobilization of thiol modified aptamers to agarose beads. The SulfoLink coupling resin contains iodoacetyl groups that react with free sulfhydryls at pH 7.5–9.0. The aptamers must be reduced prior to immobilization. This can be done with Tris (2-carboxyethyl) phosphine (TCEP), which does not interfere with the coupling and does not require removal prior to immobilization reaction.

## Materials Required

TE buffer: 10 mM Tris, 0.1 mM EDTA, pH 7.5

Folding Buffer: 1 mM MgCl<sub>2</sub>, 1X PBS, pH 7.5 (137 mM NaCl, 2.7 mM Potassium Chloride, 8 mM Sodium Phosphate dibasic, 2 mM Potassium Phosphate monobasic)

Coupling Buffer: 50 mM Tris, 5 mM EDTA-Na, pH 8.5

Wash buffer: 1 mM Tris, pH 7.5, 0.1 mM EDTA, 1 M NaCl, 1 mM MgCl<sub>2</sub>

L-Cysteine•HCl

Aptamer with thiol modification

Note: When working with complex biological media, nucleases found in the sample may fragment the aptamers. Ask us about the 3' inverted thymidine aptamer modification to protect against nucleases.

## Folding Instructions Before Immobilization (see BP: Handling & Storage for a more detailed protocol)

1. Resuspend and aliquot aptamer in TE buffer .
2. Prior to use, dilute the labeled aptamer to its working concentration in Folding Buffer.
3. Heat the aptamer solution to 85 – 95 °C for 5 minutes.
4. Allow the solution to cool to room temperature (~15 minutes).

## Buffer Considerations

Maintain buffer at pH 7.5 with 1 mM MgCl<sub>2</sub> and with similar isoelectric strength as the Folding Buffer. However, you may optimize or test the effect of certain buffer conditions on aptamer performance using control samples.

Note: Equilibrate components to room temperature before use.

## Aptamer Immobilization

Note: Perform all centrifugations at 1,000 *g* for 1 minute using a 15 mL collection tube.

### A. Prepare the sample for coupling.

1. After folding the aptamer, dilute the aptamer in Coupling Buffer to a final concentration of 50–500 ug/ mL. To reduce the disulfides, add 100  $\mu$ L  $\times$  25 mM TCEP to the mixture.
2. Incubate mixture at room temperature for 30 minutes. Equilibrate the SulfoLink column during the incubation step.

### B. Couple the aptamer to the SulfoLink column

1. Suspend the SulfoLink resin by mixing. First, remove the top cap and then the bottom cap to avoid drawing air into the resin.
2. Centrifuge to remove storage buffer.
3. Then add 2 mL of Coupling Buffer and centrifuge. Repeat this step 2 times, then replace the bottom cap.
4. Add 2-3 mL of reduced thiol-aptamer then replace the bottom and top cap and mix by rocking or end-over-end mixing at room temperature for 15 minutes. *\*Save 100  $\mu$ L to determine coupling efficiency*
5. Place the column upright and incubate at room temperature for an additional 30 minutes without mixing.
6. Remove top and bottom column caps, place column in a new tube, and centrifuge to collect non-bound aptamer. *\*Save this flow-through to determine coupling efficiency by comparing the aptamer concentrations of non-bound fraction to the starting sample and standards.*
7. Wash the column with 2 mL of Wash Buffer and centrifuge. Repeat 3 times.
8. Wash the column again with 2 mL of Coupling Buffer and centrifuge. Repeat once.

### C. Block Nonspecific Binding Sites

1. Replace the bottom cap on the column.
2. Add 15.8 mg L-Cysteine•HCl to 2 mL of Coupling Buffer to achieve a final concentration of 50 mM L-cysteine. Add the cysteine solution to the column and replace the top cap of the column.
3. Mix for 15 minutes at room temperature. Then further incubate the reaction without mixing for 30 minutes.
4. Sequentially remove the top, then bottom cap and allow column to drain.
5. The column is now ready for use.



## Perform Affinity Purification

### Materials Required

Elution Buffer: 7 M Urea

Wash/Binding Buffer: Use Folding Buffer buffer or buffer of similar isoelectric strength (see “Buffer Consideration”).

1. Remove top and bottom column caps. Add sample (<2 mL) in Wash/Binding Buffer. Allow sample to enter the resin bed, then replace bottom cap.
2. Add an additional 200  $\mu$ L of Wash/Binding Buffer, then replace top cap. Incubate column at room temperature while rocking for 30-60 minutes.
3. For the spin-purification method, remove top and bottom caps, then centrifuge the column. Without changing collection tubes, add 1 mL of Folding Buffer, and centrifuge once. Save the entire flow-through to evaluate binding efficiency and capacity.
4. To wash the resin, add 2 mL of Folding Buffer, then centrifuge. Repeat 2-4 times.

### Elute Bound Proteins

1. Elute bound proteins by adding 2 mL of Elution Buffer into column and centrifuge. Save eluted sample and repeat 2-3 times. *\*Note the Elution Buffer will denature the protein from its native conformation.*
2. Use the protein directly for SDS-PAGE or analysis by a protein assay. Or perform a buffer exchange by dialysis or gel filtration for use in a specific downstream assay or for storage.

*Note: Equilibrate the column soon after elution to prevent damage to the immobilized aptamer. Apply 4 mL of Binding/Wash Buffer and allow it to flow through the column. Cap the bottom, add 4 mL of Binding/Wash Buffer. Cap the top and store column upright at 4 °C. Do not freeze resin.*

For additional assistance contact us at [support@basepairbio.com](mailto:support@basepairbio.com) or call us at 832.230.5518

Custom Services information can be found at [www.basepairbio.com](http://www.basepairbio.com)

Catalog Aptamers can be found at [www.aptamersthatwork.com](http://www.aptamersthatwork.com)

