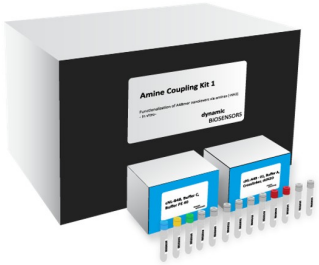

dynamic BIOSENSORS heliX plus Amine Coupling Kit 1 User Manual

dynamic BIOSENSORS

heliX +
User Manual

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heliX plus Amine Coupling Kit 1

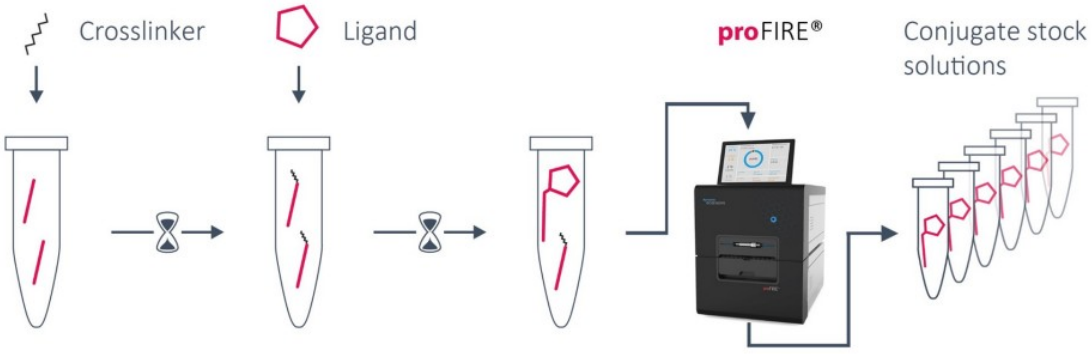
AMINE COUPLING KIT 1
Coupling of molecules with primary amines to the ligand strand – proFIRE **purification**
Dynamic Biosensors GmbH & Inc.
HK-NHS-1 v8.1

Key Features

- Allows for coupling of biomolecules with primary amines (e.g. NH2-terminus, lysines) to the Ligand strand in a single reaction tube.
- Convenient standard chemistry (NHS chemistry).
- Compatible with heliX® Adapter Chip.
- Compatible with proFIRE® purification for pure ligand-DNA conjugates (> 5 kDa).
- Coupling of multiple ligands can be performed simultaneously.
- Yields > 95 % pure ligand-DNA conjugate with user determined quality of final product.
- Includes reagents for five individual conjugation reactions (approx. 10-50 regenerations each; up to max. 500).
- Compatible with automated standard regeneration process.

Workflow Overview

3-Step Conjugation Workflow



1. DNA Modification	2. Ligand Conjugation	3. Purification	4. Ready-to-use
The Ligand strand is functionalized with a primary amine reactive NHS.	The biomolecule (ligand) is added to the functionalized Ligand strand and incubated for at least 1 h.	The Ligand strand conjugate is purified using the proFIRE® system. After buffer exchange the conjugates are aliquoted and stored.	The conjugate stock solutions are ready to use in the heliX® biosensor.

Timeline: Hands on time < 1 h | Incubation ~ 2 h | Total ~ 3 h

Product Description

Order Number: HK-NHS-1
Table 1. Contents and Storage Information

Material	Cap	Amount	Storage
Ligand strand NHS	Blue	5 x	-20°C
Buffer A [1]	Transparent	1 x 1.8 mL	-20°C
Buffer C [2]	Transparent	5 x 1.8 mL	-20°C
Buffer PE40 [3]	Transparent	5 x 1.5 mL	-20°C
ddH_2O	Transparent	1.5 mL	-20°C
Crosslinker	Brown	5 x	-20°C
Purification spin column	Red	10 x	2-8°C
2.0 mL reaction tubes for purification spin column		10 x	RT
Centrifugal filter unit (3 kDa MWCO)[4]		5 x	RT
Centrifugation collection tube		10 x	RT

Centrifugation collection tube
For research use only.
This product has a limited shelf life, please see expiry date on label.
IMPORTANT
Products may be shipped at different temperatures, but storage should adhere to the guidelines outlined in the Table.
The kit contains reagents sufficient for five conjugations of approximately 50-200 µg of biomolecule each.
The resin slurry in the purification spin column contains 0.02 % sodium azide.

Material	Comments
Benchtop microcentrifuge	Required speed range of between 1,000 x g to 13,000 x g
Vortex	
1.5 mL reaction tubes	
UV-Vis Spectrophotometer (e.g. Nanodrop)	For determination of the Ligand strand conjugate’s concentration

All necessary solutions and buffers are included in the kit.

Important Notes

a. The lyophilized Ligand strand may not always be found at the bottom of the tube; it could remain on the tube wall or in the tube cap. Please always check for the presence of the lyophilized Ligand strand, identifiable by its clear pellet appearance (you may need to remove the tube label to see it). If it is not at the bottom, please centrifuge the tube at high speed for a couple of minutes before dissolving the DNA in buffer. Alternatively, place the tip of your pipette near the DNA pellet and dispense the buffer directly onto it; the DNA will quickly dissolve.
b. The crosslinker will be linked to the primary amine groups (-NH) of the ligand. Primary amines exist at the Nterminus of each polypeptide chain and in the side-chain of lysine amino acid residues.
c. Avoid using any buffers containing primary amines (i.e. Tris, Glycine) during the conjugation process (Please check Compatibility Sheet section).
d. Up to 1 mM of Dithiothreitol (DTT) can be used during the conjugation process. Avoid using 2-Mercaptoethanol or any other thiol-based reducing agents during the conjugation process. If a reducing agent is necessary, TCEP is recommended up to 1 mM.
e. Avoid using partially purified protein samples or protein samples containing carriers (e.g. BSA).
f. To ensure the highest reaction yields, the ligand should be dissolved in Buffer C. Buffer exchange is recommended prior to the conjugation process.
g. Before starting, briefly centrifuge all tubes with blue, brown and transparent caps to ensure that all material is at the bottom of the tubes.
h. For molecules with a molecular weight around or lower than 5 kDa, extra caution is required during the purification process. Small molecules and some peptides may not be properly purified using the provided chromatographic column. For more information please email **support@dynamic-biosensors.com**.
i. If the pI of the protein is < 6, a low pH kit for conjugation (Order No: HK-NHS-3) is recommended. For more information, please email **support@dynamic-biosensors.com**.

3-Step Conjugation of a Biomolecule to a Ligand strand

Please read the entire protocol before starting and perform all steps without interruption.
TIP
This protocol can be performed simultaneously for multiple coupling reactions.
Avoid using partially purified protein samples or protein samples containing carriers (e.g., BSA).
Before starting allow the crosslinker to reach room temperature before use.
I. Nanolever Modification

1. Dissolve Ligand strand NHS in 40 µL Buffer A prior to use, vortex until all solids are completely dissolved and briefly spin down.
2. Dissolve the crosslinker (brown cap) by adding 100 µL ddH2O, vortex until all solids are completely dissolved and briefly spin down.
   IMPORTANT: Always use fresh compound.
3. Add 10 µL of the freshly prepared linker solution to one Ligand strand aliquot. Discard the remaining linker solution from step 2.
4. Vortex the reactants for 10 sec, spin down and incubate for 20 minutes at room temperature.
   IMPORTANT Do not exceed incubation time or the reaction yield will decrease.
5. In the meantime, equilibrate two purification spin columns (red cap) for one coupling reaction:
   a. Remove the column's bottom seal and loosen cap (do not remove cap).
   b. Place the column in a 2.0 mL reaction tube.
   c. Centrifuge at 1,500 × g for 1 minute to remove the storage solution.
   d. Add 400 µL of Buffer C to the column's resin bed. Centrifuge at 1,500 × g for 1 minute to remove buffer.
   e. Repeat step d and discard the resulting buffer from the reaction tube. The purification spin column should

now be in a dry state.

6. Sample loading

   a. Place the columns from step 5 in new 1.5 mL reaction tubes.

   b. Remove the cap of spin column number 1 and apply the sample from step 4 to the top of the resin bed.

   c. Centrifuge at 1,500 × g for 2 minutes to collect the sample (flow-through). Discard the Purification spin column after use.

   d. Remove the cap of spin column number 2 and apply the sample from step c to the resin bed.

   e. Centrifuge at 1,500 × g for 2 minutes to collect the sample (flow-through). Discard the Purification spin column after use.

II. Ligand Conjugation

1. Add approx. 100 µg (up to a maximum of 200 µg) of the ligand (concentration approx. 0.5 – 50 mg/mL) to the sample from step 6. For optimal conditions use a volume of approx. 50 µL.

   EXAMPLE: Adjust protein concentration to 2 mg/mL and use 50 µL for conjugation.

   IMPORTANT

   Ensure the storage buffer of the ligand does not contain any primary amines, e.g. Tris buffers, glycine (please check Important Notes).

2. Mix the reaction by pipetting up and down and let it react at room temperature for at least 1 hour.

   IMPORTANT

   Do not vortex. If necessary, the reaction can be carried out at 4 °C with a longer reaction time (e.g. overnight).

III. proFIRE® Purification

1. Perform a purification using the appropriate proFIRE® workflow (please refer to the proFIRE User Manual). Please make sure that the sample volume is 160 µL.

   a. If the volume is less than 160 µL, fill the missing volume with Buffer A.

   b. If the volume exceeds 160 µL, please perform additional 160 µL runs until all the sample is consumed.

2. Use the Data Viewer software of the proFIRE® to identify which fractions contain pure conjugate.

   An example chromatogram is shown in Additional Information section: proFIRE® purification of a Ligand strand conjugate.

3. Remove the recommended fractions from the fraction collector.

   TIP

   Do not keep the Ligand strand conjugate for prolonged time in the proFIRE®running buffer. Proceed immediately with the buffer exchange.

IV. Buffer Exchange

1. Add 500 µL of the first proFIRE® fraction containing the Ligand strand conjugate to the centrifugal filter unit. Centrifuge at 13,000 x g (up to 14,000 x g) for 10 minutes and discard flow-through.
2. Add the remaining fractions to the same filter unit and repeat the centrifugation step in order to collect all samples in one tube. (Please check Additional information: Buffer Exchange and Concentration with Centrifugal Filter Units).
3. Add 350 µL of PE40 (or TE40, HE40) buffer and centrifuge at 13,000 x g for 10 minutes. Discard the

flowthrough.

IMPORTANT

If the protein is not stable in PE40 (or TE40, HE40), please check buffer compatibility with the switchSENSE® compatibility sheet.

4. Add 350 µL of PE40 (or TE40, HE40) buffer and centrifuge at 13,000 x g for 15 minutes. Discard the flowthrough.
5. To recover the Ligand strand conjugate, place the centrifugal filter unit upside down in a new centrifugal collection tube (provided in the kit).
   Spin at 1,000 x g for 2 minutes to transfer the sample to the tube.

V. Aliquots and Storage

1. Measure the absorbance of the Ligand strand conjugate at 260 nm (= A_{260nm}) on a UV-Vis Spectrophotometer (e.g. Nanodrop).
2. Determine the concentration of the Ligand strand conjugate (c_l) by inserting (A_{260nm}) into the following equation:

$$c_l[M] = \frac{A_{260nm}}{490,000 \frac{L}{mol\ cm} \cdot d}$$

   where d is the path length (usually equal to 1 cm; however, please check the UV-Vis Spectrophotometer user manual)
3. For a ready to use solution for a biochip functionalization, please adjust the concentration to 500 nM (or up to 1 µM) with PE40 (or TE40, HE40) buffer (including up to 10 % glycerol, if needed) and prepare 20 µL aliquots.
4. Store between -86 °C and 8 °C, as desired.
   Stability of the solution is related to the stability of the ligand molecule.
   IMPORTANT
   Before a switchSENSE® interaction measurement, please add the appropriate adapter strand to the conjugate solution.

Additional Information

proFIRE® purification of a Ligand strand conjugate

1. To ensure best results from a measurement, no free Ligand strand should be present on the chip. Therefore, crude Ligand strand conjugates must be purified by ion exchange chromatography prior to measurement. This quality control step gives you additional useful information about your sample purity.
2. We recommend using the proFIRE® system equipped with an ion exchange column, Buffer A [1],and Buffer B [5] which have same composition, but different salt concentration, allowing the peak separation.
   In Figure 1 a typical proFIRE chromatogram of a Ligand strand conjugate purification is depicted, where the peak of the protein-DNA conjugate is separated from the free protein (left) and the free DNA (right).
   IMPORTANT: The proFIRE® system owns a tailored software for automatic recognition and quantitation of DNA conjugates.
3. After purification, collect the Ligand strand conjugate fractions (Figure 1: fractions 8-10), concentrate the conjugate, and exchange buffer with your buffer of choice using a Centrifugal filter unit, as described in section

II.

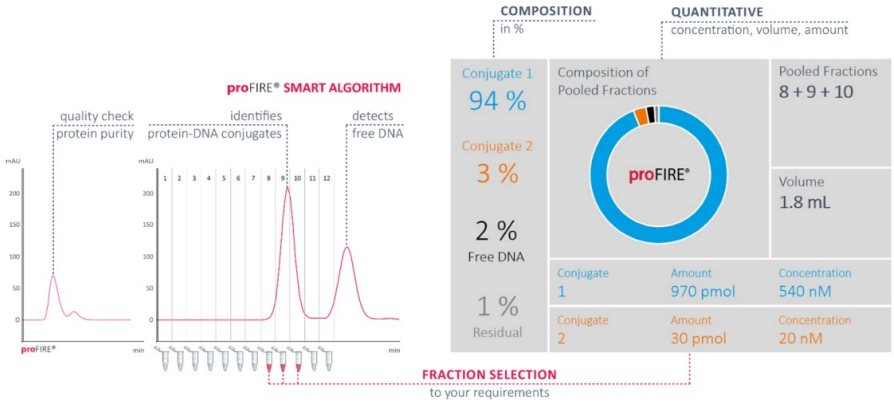
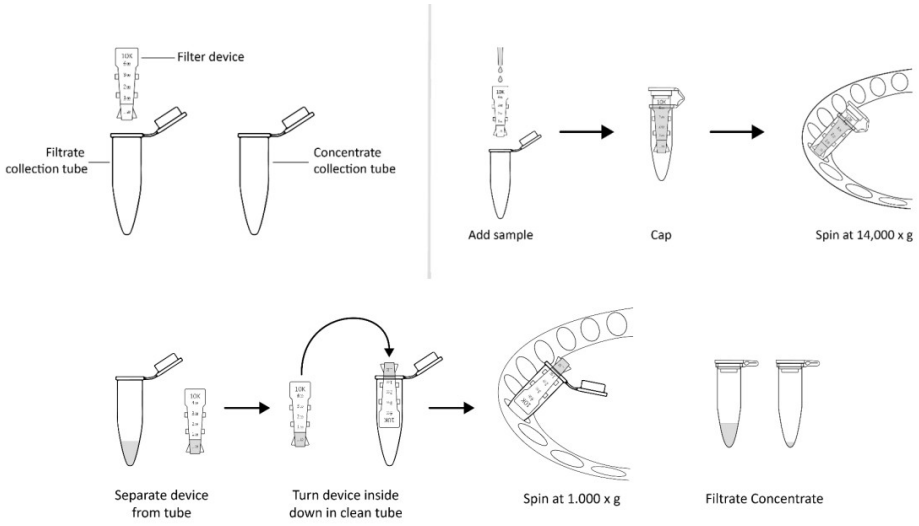


Figure 1. proFIRE® chromatogram of a ligand strand conjugate purification. Used buffers: Buffer A [1] ; Buffer B [5].
Column: DBS-chromatographic column. Flow: 1 mL/min. Used program: DNA length 48, Type 1.

Buffer Exchange and Concentration with Centrifugal Filter Units

1. Take one centrifugal filter unit, add the appropriate volume of buffer in the filter device, and cap it.
2. Place capped filter device into the centrifuge rotor, aligning the cap strap toward the center of the rotor; counterbalance with a similar device.
3. Spin the device at 13,000 x g (or 14,000 x g) for the given time.
4. Remove the flow through and repeat steps 1-3.
5. Remove the assembled device from the centrifuge and separate the filter device from the microcentrifuge tube.
6. To recover the conjugate, place the filter device upside down in a clean centrifugal tube, aligning open cap towards the center of the rotor; counterbalance with a similar device. Spin for 2 minutes at 1,000 x g to transfer the sample from the device to the tube.



Compatibility Sheet

Buffer additives

The conjugation of ligands with all available coupling kits can be performed with many different additives. The following list shows all tested ones, but please note that others not listed here may also be successfully used.

Additive	Up to	Suitability Amine Coupling	Suitability Thiol Coupling
EDTA	1 mM	●○○	●○○
DTT*	1 mM	●○○	○○●
TCEP	1 mM	●○○	●○○
Tris**	1 mM	○○●	●○○
DMSO	2 %	●○○	●○○
ATP	0.5 mM	●○○	●○○
$MgCl_2$	2.5 mM	●○○	●○○
Glycine**	—	○○●	○●○
Mannitol	8 %	●○○	●○○
Glycerol	10 %	●○○	●○○
Trehalose	8 %	●○○	●○○
Histidin**	30 mM	○○●	○●○
Acetonitrile***	50 %	●○○	●○○
Trifluoroacetic acid	0.1 %	●○○	●○○

* thiol-based reducing agents
** contains primary amines
*** caution, may harm the ligand

pH/pI
The pH value for the conjugation buffer may range from pH 5.0 to pH 8.0, depending on the ligand characteristics. When performing a conjugation of proteins with a pI of < 6, please note that using a buffer with lower pH may result in a better yield of conjugate.

Buffer	**pH**	**Order No**	**Composition**
Phosphate-Citrate Buffer	pH 5	–	50 mM buffer salt, 150 mM NaCl
Buffer M	pH 6.5	BU-M-150-1	50 mM MES, 150 mM NaCl
Buffer A	pH 7.2	BU-P-150-10	50 mM Na_2HPO_4/NaH_2PO_4, 150 mM NaCl
Buffer C	pH 8.0	BU-C-150-1	50 mM Na_2HPO_4/NaH_2PO_4, 150 mM NaCl

Salt concentration
For standard conjugations, 50 mM buffer salt and 150 mM NaCl (monovalent salt) are used.
When performing conjugation of strongly charged ligands, make sure that the concentration of NaCl is sufficiently high (up to 400 mM NaCl is recommended). Otherwise, precipitation of DNA may occur.
The shielding effect of monovalent sodium cations leads to DNA stabilization through neutralization of the negative charge on the sugar phosphate backbone.

Useful Order Numbers

Table 3. Order Numbers

Product Name	Amount	Order No
heliX® Amine coupling kit 3 (low pI biomolecules)	5 conjugations	HK-NHS-3
heliX® Thiol coupling kit 1	5 conjugations	HK-MAL-1
Centrifugal filter unit (3 kDa MWCO)	5 pcs.	CF-003-5
Centrifugal filter unit (10 kDa MWCO)	5 pcs.	CF-010-5
10x Buffer A [1]	50 mL (yielding 500 mL)	BU-P-150-10
5x Buffer B [5]	50 mL (yielding 250 mL)	BU-P-1000-5
1x Buffer C [2]	12 mL	BU-C-150-1
1x Buffer M [6]	50 mL	BU-M-150-1

My Notes

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Instruments and chips are engineered and manufactured in Germany.

[1] Buffer A: 50 mM Na2HPO./NaH2PO,, 150 mM NaCl, pH 7.2
[2] Buffer C: 50 MM Na,HPO,/NaH,PO,, 150 mM NaCl, pH 8.0
[3] Buffer PE40: 10 mM Na,HPO,/NaH,PO,, 40 mM NaCl, pH 7.4, 0.05 % Tween, 50 UM EDTA, 50 uM EGTA
[4] For conjugation of proteins with a molecular weight higher than 20 kDa: Centrifugal filter units with a MWCO of 10 kDa can be ordered for a faster concentration process (Order No: CF-010-5).
[5] Buffer B: 50 mM Na,HPO,/NaH,PO,, 1 M NaCl, pH 7.2
[6] Buffer M: SO mM MES, 150 mM NaCl, pH 6.5

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