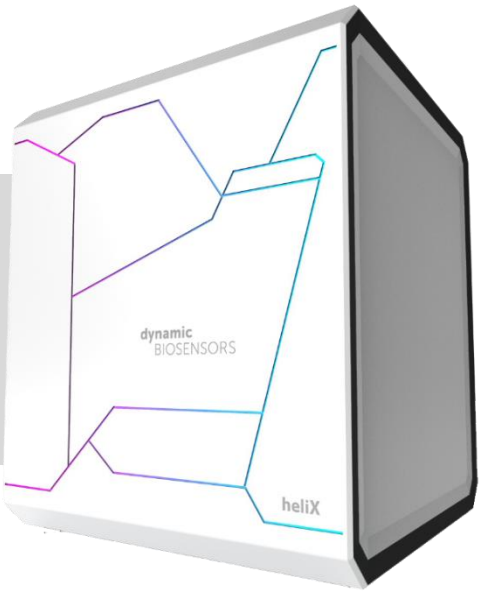
USER MANUAL
HK-NHS-3

dynamic BIOSENSORS

heliX® Amine Coupling Kit 3

Functionalization of the ***Ligand strand*** with biomolecules containing a primary amine

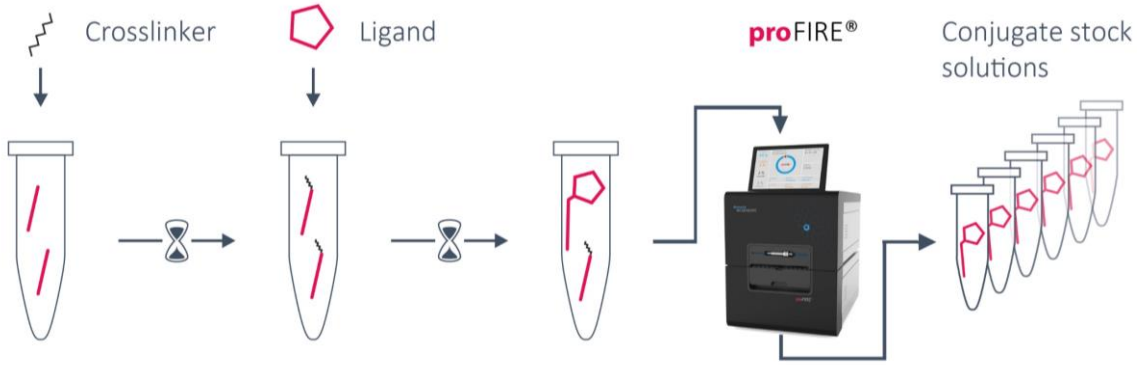


Key Features

- 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)
- Applicable for biomolecules with a low pI (pI < 6)
- Compatible with all **switch**SENSE® *adapter and multipurpose chips*
- Compatible with **pro**FIRE® 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

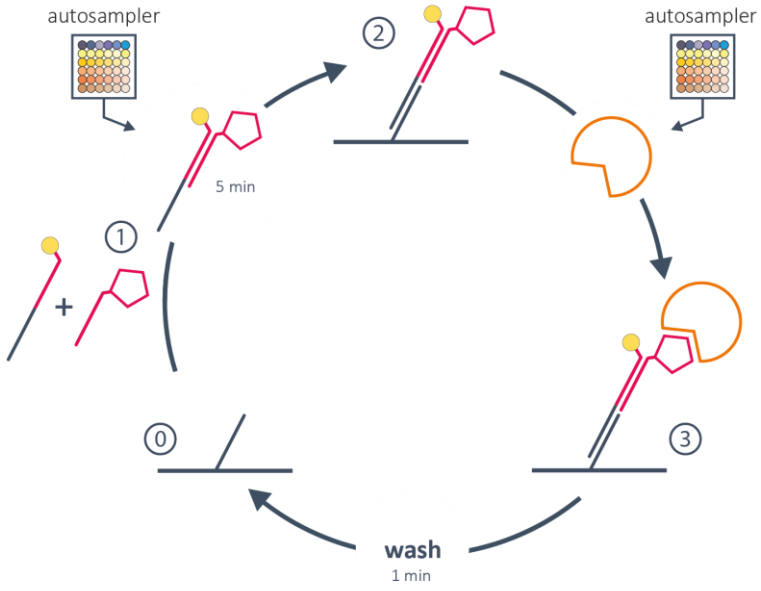
3-Step Conjugation Workflow



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 **pro**FIRE® system. After buffer exchange the conjugates are aliquoted and stored.	The conjugate stock solutions are ready for insertion in the **heliX**® instruments.

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

Measurement Workflow with Conjugated ***Ligand strands***



⓪→①→②

Automatic functionalization of the **switch**SENSE® chip with ***Ligand strand*** conjugate prehybridizied with Adapter strand.

②→③

Measurement of ligand-analyte Interaction by flowing analyte solution (association) or buffer solution (dissociation) over the chip.

③→⓪

Washing away of the ***Ligand strand*** Adapter analyte complex from the surface by DNA denaturation under basic pH conditions, ensuring a complete removal of the analyte.

Product Description

Order Number **HK-NHS-3**

TABLE 1 | Contents and Storage Information

Material	Cap	Amount	Storage	Comment
Ligand strand NHS	blue	5 x	-20°C	
Buffer A (50 mM Na_2HPO_4/NaH_2PO_4 pH 7.2, 150 mM NaCl)	trans-parent	1 x 1.8 mL	-20°C	
Buffer M (50 mM MES pH 6.5, 150 mM NaCl)	trans-parent	5 x 1.8 mL	-20°C	
Buffer PE40 (10 mM Na_2HPO_4/NaH_2PO_4 pH 7.4, 40 mM NaCl, 0.05 % Tween, 50 µM EDTA, 50 µM EGTA)	trans-parent	5 x 1.5 mL	-20°C	
ddH_2O	trans-parent	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	r.t.	
Centrifugal filter unit (3 kDa MWCO)[1]		5 x	r.t.	
Centrifugation collection tube		10 x	r.t.	

For *in vitro* use only.

Please check date of expiry on the kit prior to use. Products are shipped at ambient temperature. The kit contains reagents sufficient for 5 conjugations of approx. 50-200 µg biomolecule each. The resin slurry of the Purification spin column contains 0.02 % sodium azide.

[1] 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 (Please see page 10 for order number).

Additional Materials Required

TABLE 2 | Additional Materials.

Material	Comment
Benchtop microcentrifuge	Required speed range of between 1,000 x g to 13,000 x g
Vortexer	
1.5 mL reaction tubes	
UV-Vis spectroscopy (e.g. Nanodrop)	For concentration determination of the conjugate

All necessary solutions and buffers are included in the kit.

Important Notes

- The crosslinker will be linked to the primary amine groups (-NH_2) of the ligand. Primary amines exist at the N-terminus of each polypeptide chain and in the side-chain of lysine amino acid residues.
- Avoid using any buffers containing primary amines (i.e. Tris, Glycine) during the conjugation process.
- 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. For reducing agents during interaction measurement, please refer to the **switch**SENSE® compatibility sheet (application area on **www.dynamic-biosensors.com/switchsense**).
- Avoid using partially purified protein samples or protein samples containing carriers (e.g. BSA).
- To ensure highest reaction yields, the ligand should be dissolved in Buffer M. Buffer exchange is recommended prior to the conjugation process[1].
- Before you begin, briefly centrifuge all tubes with blue, brown and transparent caps to ensure that all material is at the bottom of the tubes.
- For molecules with a molecular weight around or lower than 5 kDa, special care during the purification process should be taken. Small molecules and some peptides may not be properly purified using the provided chromatographic column. For more information please email **support@dynamic-biosensors.com**.

[1] See page 10 for order no.

3-Step Conjugation of a Biomolecule to a *Ligand strand* in a Reaction Tube

Please read the entire protocol before starting and **perform all steps without interruption**.

TIP: This protocol can be performed simultaneously for multiple coupling reactions.

Before you begin: 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** ddH_2O, vortex until all solids are completely dissolved and briefly spin down. **IMPORTANT:** *Always use fresh compounds.*

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 M** 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 x g for 2 min 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 x g for 2 min to collect the sample (flow-through). Discard the Purification spin column after use.

II Ligand Conjugation

7. 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 see page 4, Important Notes).*

8. 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

Please refer to the **pro**FIRE® User Manual.

9. Perform a purification using the appropriate **pro**FIRE® workflow. Please make sure that the sample volume is 160 µL.
   - If the volume is less than 160 µL, make up the missing volume with Buffer M.
   - If the volume exceeds 160 µL, please perform additional 160 µL runs until all of the sample is consumed.

10. Use the Data Viewer software of the **pro**FIRE® to identify which fractions contains pure conjugate. On page 8 (Additional Information section: **pro**FIRE® purification of a ***Ligand strand*** conjugate) an example chromatogram is shown.

11. Remove the recommended fractions from the fraction collector.

IV Buffer Exchange

12. a. Add **500 µL** of the first **pro**FIRE ® 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.

    b. 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 on page 9: Additional information for the right use of centrifugal filter unit).

    c. Add **350 µL of PE40** (or TE40, HE40) buffer and centrifuge at 13,000 x g for **10 minutes**. Discard the flow-through again.

    If the protein is not stable in **PE40** (or TE40, HE40), please check buffer compatibility with the **switch**SENSE® compatibility sheet (Application area on **www.dynamic-biosensors.com/switchsense**).

    d. Add **350 µL of PE40** (or TE40, HE40) buffer and centrifuge at 13,000 x g for **15 minutes**. Discard the flow-through again.

    e. To recover the ***Ligand strand*** conjugate, place the centrifugal filter unit upside down in a new centrifugal collection tube (provided in the kit).
Spin for **2 minutes** at 1,000 x g to transfer the sample to the tube.

Optional: *Concentration*

Check ***Ligand strand*** conjugate concentration after buffer exchange by using absorbance at 260 nm and the following equation:

c (*Ligand strand* conjugate)= $A_{260\ nm}$/(490,000 L mol^{-1} cm^{-1} * d)

d = optical path length
(usually d = 1 cm, please check photometer manual for further information).

V Aliquots and Storage

13. Adjust the concentration to **200 nM – 1 µM** with **PE40** (or TE40, HE40) buffer (including up to 10 % glycerol if needed) and prepare **20 µL** aliquots.

14. Store between 8 °C and -86 °C as desired.

IMPORTANT: *Prior to an interaction measurement please add the appropriate adapter strand to the conjugate solution.*

Additional Information

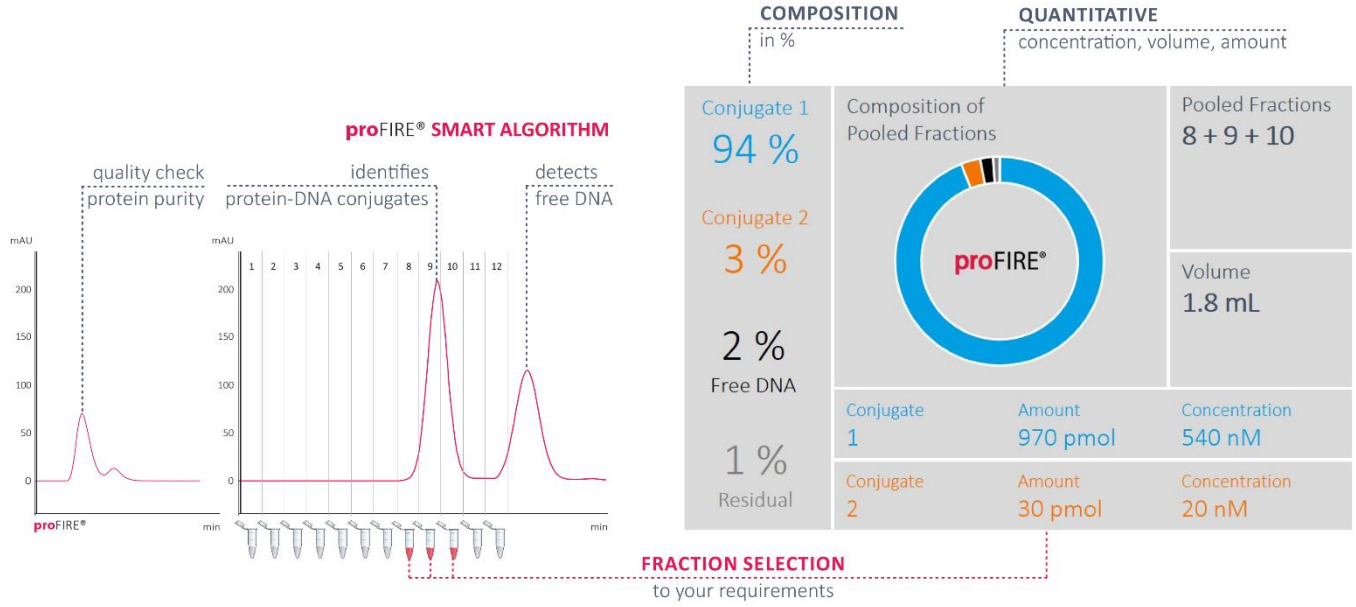
I proFIRE® purification of a *Ligand strand* conjugate

1. To ensure best results from a measurement no free ***Ligand strands*** 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 provided **pro**FIRE® system equipped with an ion exchange column. For an example chromatogram, see figure below.

   Prepare 250 mL Buffer A (50 mM Na_2HPO_4/ NaH_2PO_4 pH 7.2 and 150 mM NaCl)[1] and 250 mL Buffer B (50 mM Na_2HPO_4/NaH_2PO_4 pH 7.2 and 1 M NaCl)[1].

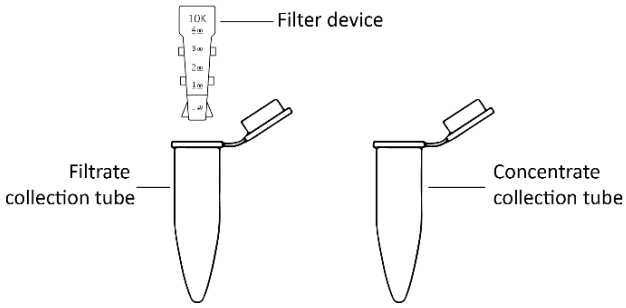
3. Collect the ***Ligand strand*** conjugate fraction (here: 8-10), concentrate the conjugate and exchange buffer with your buffer of choice using a Centrifugal filter unit, as described in section II (Additional information, page 9).

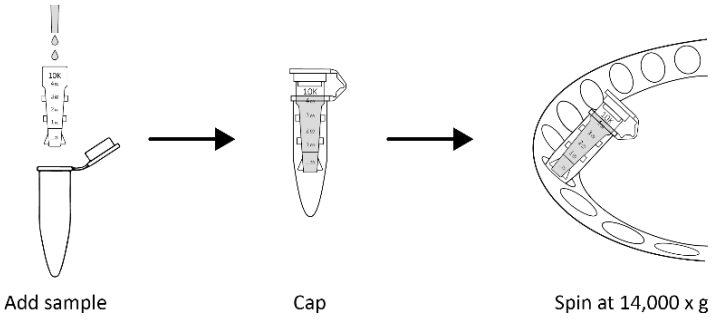


proFIRE® chromatogram of a *Ligand strand* conjugate purification.
Used buffers: Buffer A: 50 mM Na_2HPO_4/NaH_2PO_4 pH 7.2, 150 mM NaCl; Buffer B: 50 mM Na_2HPO_4/NaH_2PO_4 pH 7.2, 1 M NaCl.
Column: DBS-Chromatographic column. *Flow:* 1 mL/min.
Used program: DNA length **48**, *Type* **1**.

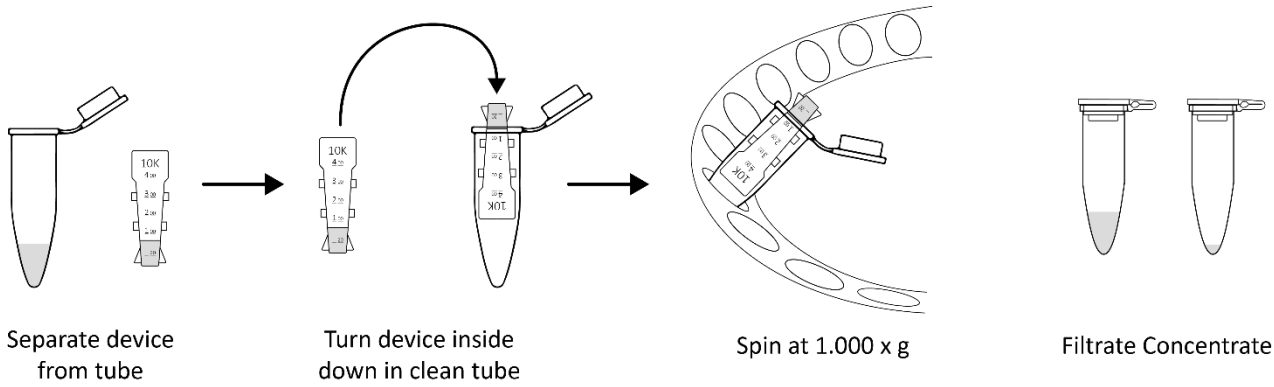
[1] See page 10 for order no.

II 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 flowthrough 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.



Useful Order Numbers

TABLE 3 | Order Numbers.

Product name	Order Number
heliX® Amine coupling kit 1 (**pro**FIRE® purification)	HK-NHS-1
heliX® Amine coupling kit 2 (spin column purification)	HK-NHS-2
heliX® Amine coupling kit 3 (low pI biomolecules)	HK-NHS-3
Centrifugal filter unit (3 kDa MWCO), 5 pcs.	CF-003-5
Centrifugal filter unit (10 kDa MWCO), 5 pcs.	CF-010-5
Chromatographic column	TB-CC-1-1
1x Buffer MES pH 6.5 (12 mL of: 50 mM MES, 150 mM NaCl)	BU-M-150-1
10x Buffer A pH 7.2 (50 mL of: 500 mM Na_2HPO_4/NaH_2PO_4, 1.5 M NaCl) Yields 0.5 L of: 50 mM Na_2HPO_4/NaH_2PO_4, 150 mM NaCl	BU-P-150-10
5x Buffer B pH 7.2 (50 mL of: 250 mM Na_2HPO_4/NaH_2PO_4, 5 M NaCl) Yields 0.25 L of: 50 mM Na_2HPO_4/NaH_2PO_4, 1 M NaCl	BU-P-1000-5

My Notes

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Instruments and biochips are engineered and manufactured in Germany.