dynamic BIOSENSORS HK-NHS-1 Helix Amine Coupling Kit User Manual

Contents

dynamic BIOSENSORS

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Product Information

- **Specifications:**
  - **Order Number:** HK-NHS-1

Contents and Storage Information:

Material	Amount	Comment
Ligand strand	5 x	For in vitro use only.

Product Usage Instructions

- **Conjugation Workflow:**
  - **DNA Modification:** Follow the protocol provided for nanolever modification.
- **Ligand Conjugation:**
  - Add the biomolecule (ligand) to the functionalized Ligand strand.
  - Incubate for at least 1 hour at room temperature.
  - **Purification:** Equilibrate two purification spin columns for one coupling reaction.
  - **Ready-to-use:** The conjugate stock solutions are ready for instruments.
- **Measurement Workflow:**
  - Measure the ligand-analyte interaction by flowing analyte solution (association) or buffer solution (dissociation) over the chip.
  - Wash away the Ligand strand Adapter analyte complex from the surface by DNA denaturation under basic pH conditions.
- **Timeline:**
  - Hands-on time < 1 h | Incubation ~ 2 h | Total ~ 3 h

FAQs

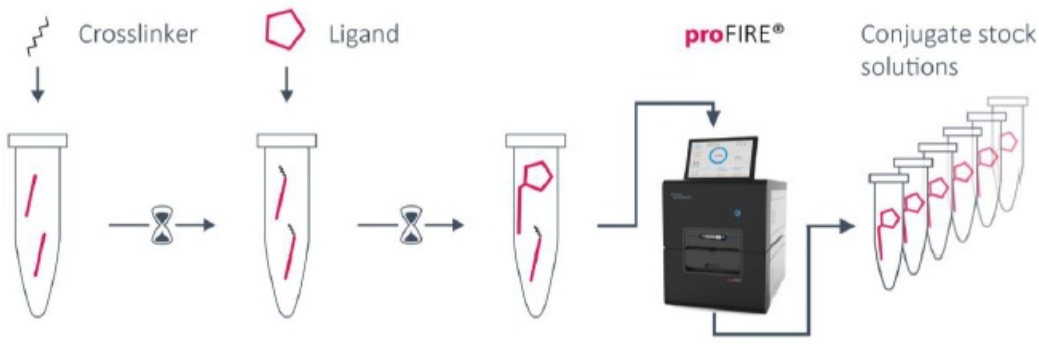
- **Question: Can I use the kit for any other purposes?**
  - **Answer:** The kit is designed for in vitro use only and should not be used for other purposes.

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 all switchSENSE® adapters and multipurpose chips
- Compatible with proFIRE® purification for pure ligand-DNA conjugates (> 5 kDa)
- The 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

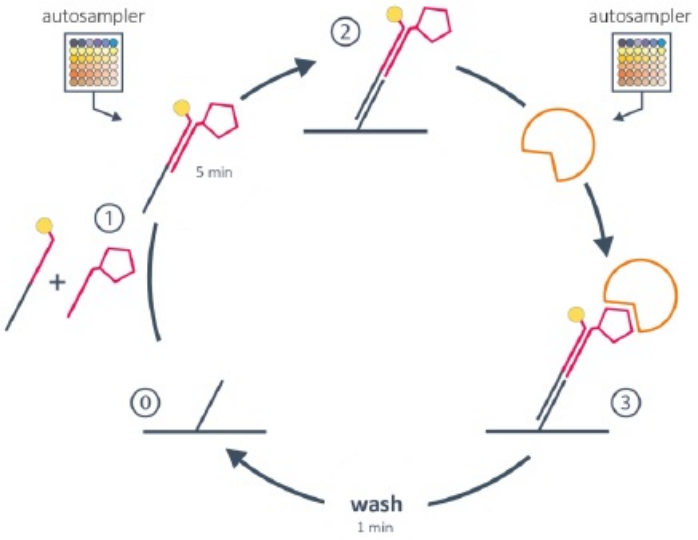


1. **DNA Modification**
   - The Ligand strand is functionalized with a primary amine-reactive NHS.
2. **Ligand Conjugation**
   - The biomolecule (ligand) is added to the functionalized Ligand strand and incubated for at least 1 h.
3. **Purification**
   - The Ligand strand conjugate is purified using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

Measurement Workflow with Conjugated Ligand strands

- →①→② Automatic functionalization of the switchSENSE® chip with Ligand strand conjugate prehybridized with Adapter strand.
- ②→③ Measurement of ligand-analyte Interaction by flowing analyte solution (association) or buffer solution (dissociation) over the chip.

- ③→ Washing away 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-1

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- par ent	1 x 1.8 mL	-20°C	
Buffer C (50 mM Na_2HPO_4/NaH_2PO_4 pH 8.0, 150 mM NaCl)	trans- par ent	5 x 1.8 mL	-20°C	
Buffer PE40 (10 mM Na_2HPO_4/NaH_2PO_4 pH 7.4, 4 0 mM NaCl, 0.05 % Tween, 50 µM EDTA, 50 µM E GTA)	trans- par ent	5 x 1.5 mL	-20°C	
ddH2O	trans- par ent	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 the date of expiry on the kit before 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.

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 (-NH2) 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 switchSENSE® 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 C. Buffer exchange is recommended before the conjugation process1.
- 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@dynamicbiosensors.com**.
- If the pI of the protein is < 6, a low pH kit for conjugation is recommended.1 For more information, please email **support@dynamic-biosensors.com**.

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.

Nanolever Modification

1. Dissolve Ligand strand NHS in 40 µL Buffer A before use, vortex until all solids are completely dissolved and briefly spin down.
2. Dissolve the crosslinker (brown cap) by adding 100 µL ddH2O, and 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 the cap (do not remove the 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 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.

    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, or 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).

    proFIRE® Purification
    - Please refer to the proFIRE® User Manual.
9. Perform a purification using the appropriate proFIRE® 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 C.
    - If the volume exceeds 160 µL, please perform an additional 160 µL runs until all of the sample is consumed.
10. Use the Data Viewer software of the proFIRE® to identify which fractions contain pure conjugate. On page 8 (Additional Information section: proFIRE® purification of a Ligand strand conjugate) an example chromatogram

is shown.

11. Remove the recommended fractions from the fraction collector.

    Buffer Exchange

    - **a.** Add 500 µL of the first profile ® 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 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 switchSENSE® compatibility sheet (Application area on **www.dynamicbiosensors.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)= A260 nm/(490,000 L mol-1 cm-1 * d)
    - **d =** optical path length (usually d = 1 cm, please check the photometer manual for further information).

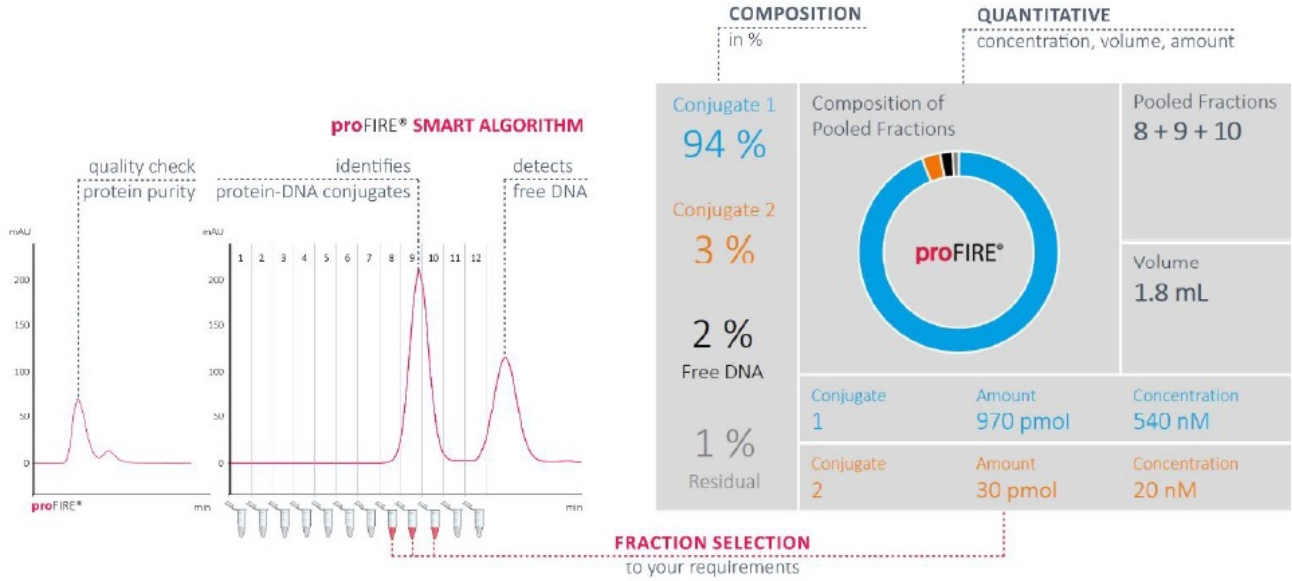
    Aliquots and Storage

12. 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.
13. Store between 8 °C and -86 °C as desired.
    - **IMPORTANT:** Before an interaction measurement please add the appropriate adapter strand to the conjugate solution.

Additional Information

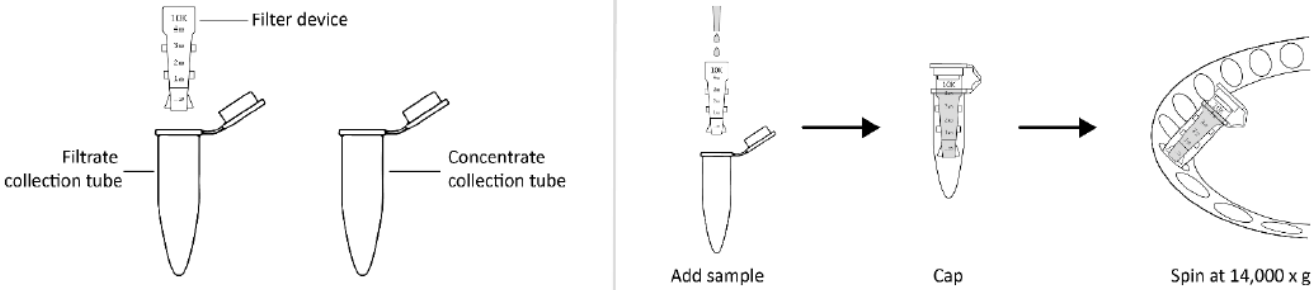
proFIRE® purification of a Ligand strand conjugate

1. To ensure best results from a measurement no free Ligand strands should be present on the chip. Therefore crude Ligand strand conjugates must be purified by ion exchange chromatography before measurement. This quality control step gives you additional useful information about your sample purity.
2. We recommend using the provided proFIRE® system equipped with an ion exchange column. For an example chromatogram, see the figure below. Prepare 250 mL Buffer A (50 mM Na2HPO4/ NaH2PO4 pH 7.2 and 150 mM NaCl)1 and 250 mL Buffer B (50 mM Na2HPO4/NaH2PO4 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).

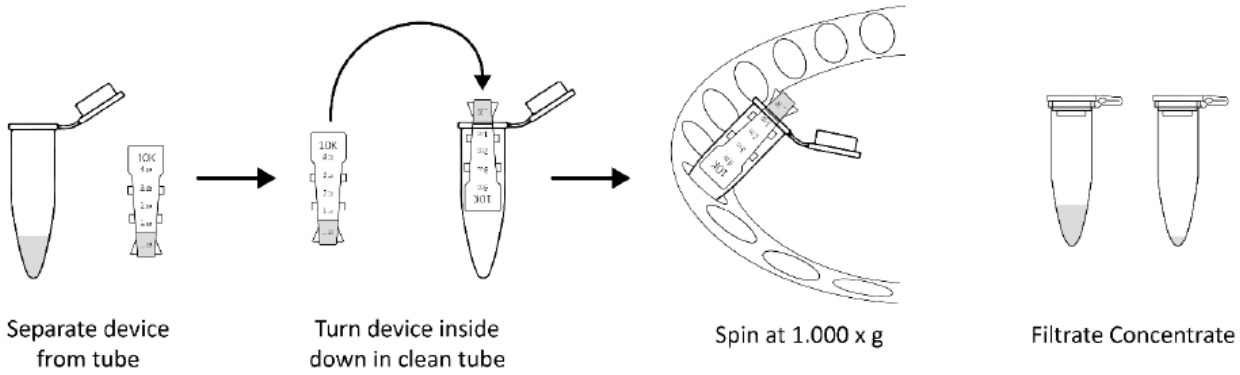


- proFIRE® chromatogram of a Ligand strand conjugate purification.
- **Used buffers:**
  - **Buffer A:** 50 mM Na2HPO4/NaH2PO4 pH 7.2, 150 mM NaCl;
  - **Buffer B:** 50 mM Na2HPO4/NaH2PO4 pH 7.2, 1 M NaCl.
- **Column:** DBS-Chromatographic column.
  - **Flow:** 1 mL/min.
  - **Used program:** DNA length 48, Type 1.

Buffer Exchange and Concentration with Centrifugal Filter Units



1. Take one centrifugal filter unit, add the appropriate volume of buffer to the filter device, and cap it.
2. Place capped filter device into the centrifuge rotor, aligning the cap strap toward the centre 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 the open cap towards the centre 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 (proFIRE® 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 C pH 8.0 (12 mL of: 50 mM Na2HPO4/NaH2PO4, 150 mM NaCl)	BU-C-150-1
10x Buffer A pH 7.2 (50 mL of: 500 mM Na2HPO4/NaH2PO4, 1.5 M NaCl) Yields 0.5 L of 50 mM Na2HPO4/NaH2PO4, 150 mM NaCl	BU-P-150-10
5x Buffer B pH 7.2 (50 mL of: 250 mM Na2HPO4/NaH2PO4, 5 M NaCl) Yields 0.25 L of 50 mM Na2HPO4/NaH2PO4, 1 M NaCl	BU-P-1000-5

Contact

- **Dynamic Biosensors GmbH**
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  - Munich
  - Germany
- **Dynamic Biosensors,**
  - Inc. 300 Trade Center,

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- **Order Information** order@dynamic-biosensors.com.
- **Technical Support** support@dynamic-biosensors.com.
- www.dynamic-biosensors.com.
- **Get it on** Google Play.
- **Download on the** App Store.

 User Manual_HK-NHS-1_v8.0 www.dynamic-biosensors.com. Functionalization of the Ligand strand with biomolecules containing a primary amine www.dynamic-biosensors.com.

Documents / Resources

	dynamic BIOSENSORS HK-NHS-1 Helix Amine Coupling Kit [pdf] User Manual HK-NHS-1 Helix Amine Coupling Kit, HK-NHS-1, Helix Amine Coupling Kit, Amine Coupling Kit, Coupling Kit, Kit

References

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