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BRUKER heliX plus Thiol Coupling Kit 1

Specifications

- Product Name: heliX + THIOL COUPLING KIT 1
- Order Number: HK-MAL-1
- Manufacturer: Dynamic Biosensors GmbH
- Version: v6.1

Product Description

- The heliX + THIOL COUPLING KIT 1 from Dynamic Biosensors GmbH is designed for conjugating biomolecules to a ligand strand.
- The kit includes all necessary materials for the conjugation process, including ligand strand, buffers, crosslinker, purification tools, and reaction tubes.

Key Features

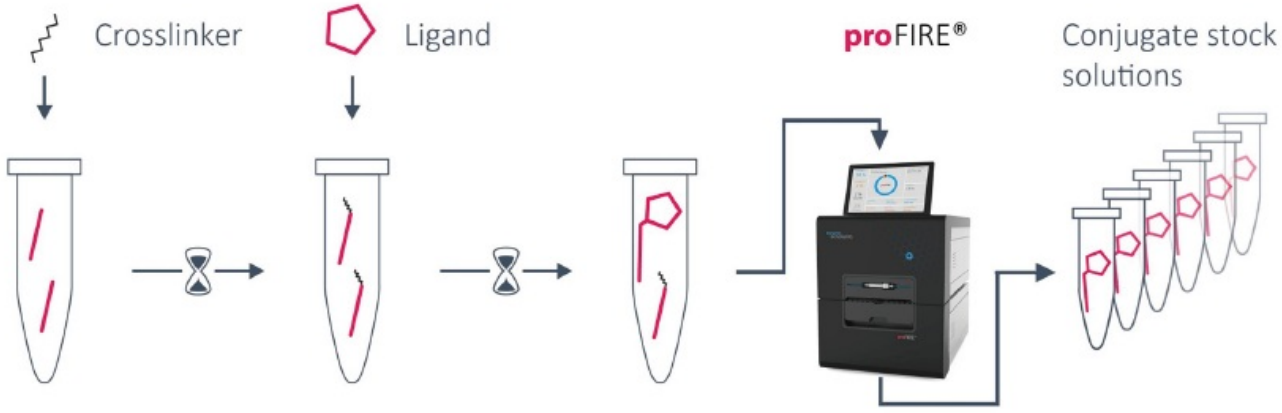
- Allows for coupling of biomolecules with free thiols (e.g., cysteines) to the Ligand strand in a single reaction tube.
- Convenient standard chemistry (Maleimide chemistry).
- Compatible with heliX® Adapter Chip and multipurpose chips.
- Compatible with proFIRE® purification for pure ligand-DNA conjugates (> 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 the 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 free thiol reactive groups.	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-MAL-1

Table 1. Contents and Storage Information

Material	Cap	Amount	Storage
Ligand strand MAL	Blue	5 x	-20°C
Buffer A [1]	Transparent	5 x 1.8 mL	-20°C
Buffer C [2]	Transparent	1 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	Green	5 x	-20°C
Purification spin column	Red	10 x	2-8°C
2.0 mL reaction tubes for purification sp in 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 the expiry date on the label.

IMPORTANT

- Products might be shipped at different temperatures, but the storage should respect the indications reported in Table 1.
- 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	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 the determination of the *Ligand strand* conjugate's concentration

All necessary solutions and buffers are included in the kit.

Important Notes

- The lyophilized Ligand strand is not always found at the bottom of the tube, but may remain on the tube wall or in the tube cap. Please, always check the presence of the lyophilized Ligand strand, which has the appearance of a clear pellet (it may be necessary to remove the tube label to see it). If it is not on the bottom, please spin down (at high speed) the tube for couple of minutes before dissolving the DNA in buffer or, in alternatively, place the tip of your pipette in proximity of the DNA pellet and dispense the buffer directly on it; the DNA will quickly dissolve.
- The crosslinker will be linked to the free thiol groups of the ligand.
- Do not use 2-Mercaptoethanol or other thiol-based reducing agents during the conjugation process. If a reducing agent is necessary, TCEP is recommended up to 1 mM.
- Avoid using partially purified protein samples or protein samples containing carriers (e.g. BSA).
- To ensure the highest reaction yields, the ligand should be dissolved in Buffer A. Buffer exchange is recommended prior to the conjugation process.
- Before starting, briefly centrifuge all tubes with blue, green, 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, 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.
- If the pI of the protein is < 6, a low pH buffer for conjugation (Order No: BU-M-150-1) 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.

- Nanolever Modification
- Dissolve Ligand strand MAL in 40 µL Buffer C prior to use, vortex until all solids are completely dissolved, and briefly spin down.
- Dissolve the crosslinker (green cap) by adding 100 µL ddH2O, vortex until all solids are completely dissolve, and briefly spin down. IMPORTANT: Always use fresh compound.
- Add 10 µL of the freshly prepared linker solution to one Ligand strand aliquot. Discard the remaining linker solution from step 2.
- Vortex the reactants for 10 sec, spin down, and incubate for 45 minutes at room temperature.
  IMPORTANT Do not exceed incubation time or the reaction yield will decrease.
- In the meantime, equilibrate two purification spin columns (red cap) for one coupling reaction:
  - Remove the column's bottom seal and loosen cap (do not remove cap).
  - Place the column in a 2.0 mL reaction tube.
  - Centrifuge at 1,500 × g for 1 minute to remove the storage solution.
  - Add 400 µL of Buffer A to the column's resin bed. Centrifuge at 1,500 × g for 1 minute to remove buffer.

    - Repeat step d and discard the resulting buffer from the reaction tube. The purification spin column should now be in a dry state.
- Sample loading
    - Place the columns from step 5 in new 1.5 mL reaction tubes.
    - Remove the cap of spin column number 1 and apply the sample from step 4 to the top of the resin bed.
    - Centrifuge at 1,500 × g for 2 minutes to collect the sample (flow-through). Discard the Purification spin column after use.
    - Remove the cap of spin column number 2 and apply the sample from step c to the resin bed.
    - Centrifuge at 1,500 × g for 2 minutes to collect the sample (flow-through). Discard the Purification spin column after use.

Ligand Conjugation

- 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 approximately. 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 thiols, e.g., 2-Mercaptoethanol (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).

proFIRE® Purification

- 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.
- If the volume is less than 160 µL, fill the missing volume with Buffer A.
- If the volume exceeds 160 µL, please perform additional 160 µL runs until all the sample is consumed.
- Use the Data Viewer software of the proFIRE® to identify which fractions contain pure

conjugate. An example chromatogram is shown in Athe Additional Information section: proFIRE® purification of a Ligand strand conjugate.
- Remove the recommended fractions from the fraction collector.

TIP

- Do not keep the Ligand strand conjugate too long in the proFIRE® running buffer. Proceed immediately with the buffer exchange.

Buffer Exchange

- 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.
- 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).
- Add 350 µL of PE40 (or TE40, HE40) buffer and centrifuge at 13,000 x g for 10 minutes. Discard the flow-through.
    - **IMPORTANT:** If the protein is not stable in PE40 (or TE40, HE40), please check buffer compatibility with the switchSENSE® compatibility sheet.
- Add 350 µL of PE40 (or TE40, HE40) buffer and centrifuge at 13,000 x g for 15 minutes. Discard the flow-through.
- 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.

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 ( ) 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)
- 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.
- Store between -86 °C and 8 °C, as desired. The 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

- 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.
- 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.
- 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.

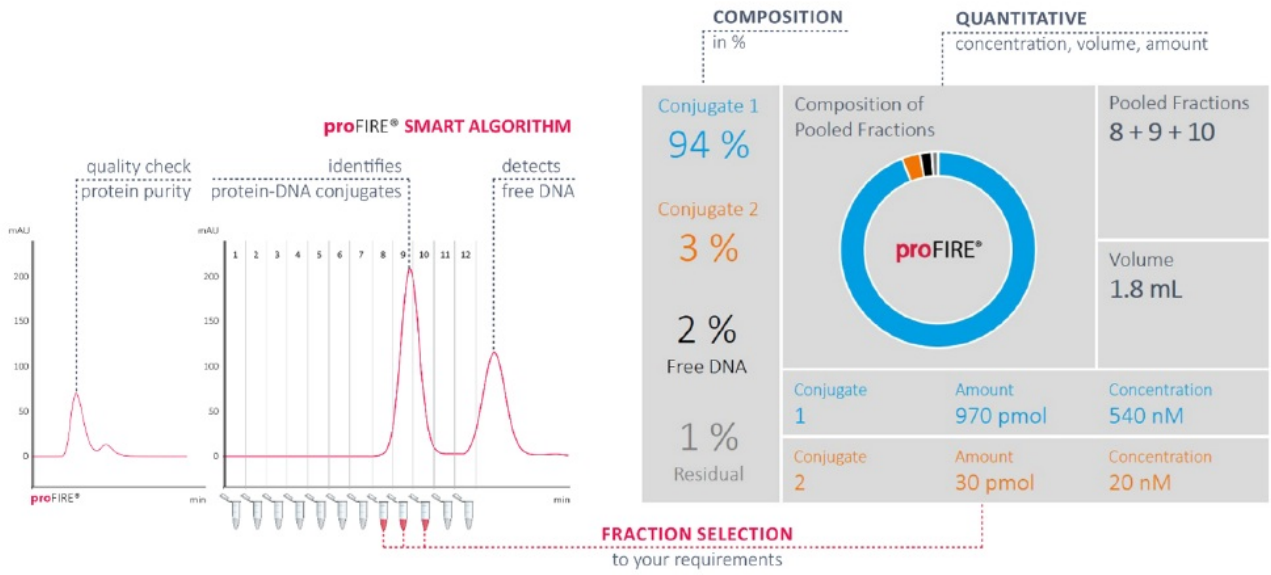
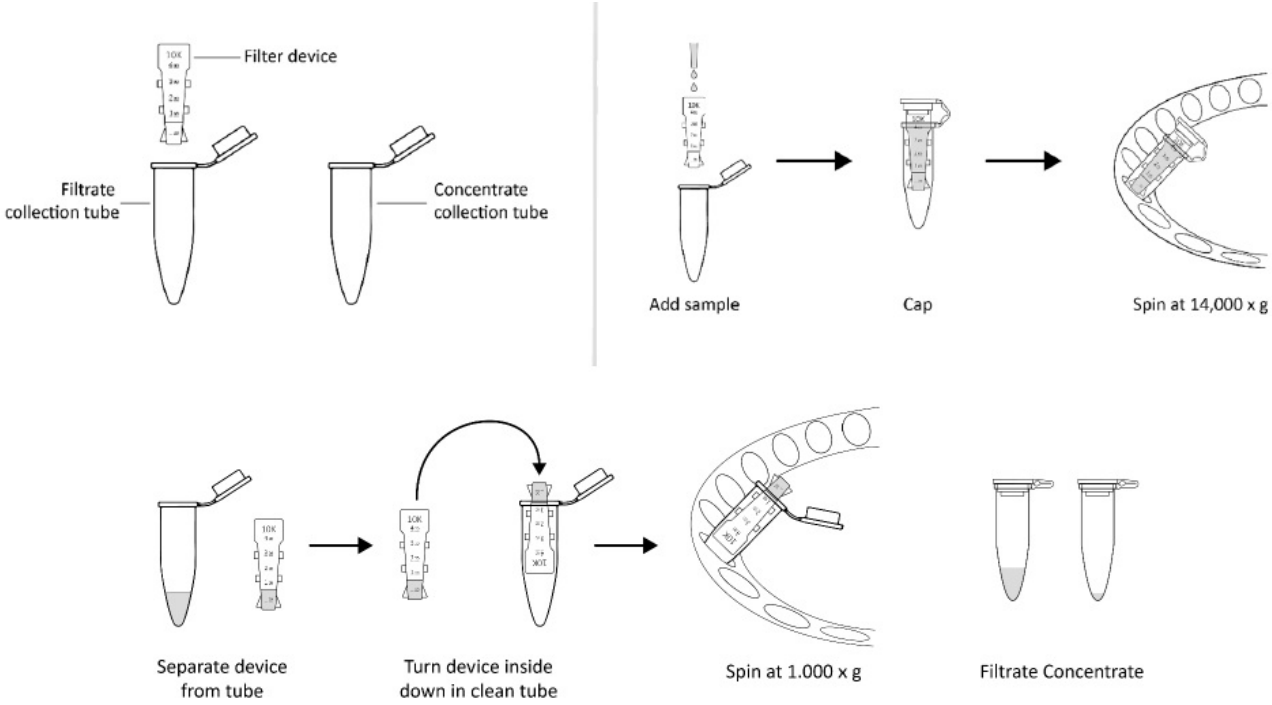


Figure 1. proFIRE® chromatogram of a ligand strand conjugate purification. Used buffers: Buffer A [1]; Buffer B [5]. Column: proFIRE column. Flow: 1 mL/min. Used program: DNA length 48, Type 1.

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 the 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 the 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 a 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 1	5 conjugations	HK-NHS-1
heliX® Amine coupling kit 3 (low pI biomolecules)	5 conjugations	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
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 M [6]	50 mL	BU-M-150-1

Contact

- Dynamic Biosensors GmbH
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- Germany
- Bruker Scientific LLC
- 40 Manning Road, Manning Park
- Billerica, MA 01821
- USA
- Order Information order@dynamic-biosensors.com
  Technical Support support@dynamic-biosensors.com
- www.dynamic-biosensors.com

1. Buffer A: 50 mM Na2HPO4/NaH2PO4, 150 mM NaCl, pH 7.2

2. Buffer C: 50 mM Na2HPO4/NaH2PO4, 150 mM NaCl, pH 8.0
3. Buffer PE40: 10 mM Na2HPO4/NaH2PO4, 40 mM NaCl, pH 7.4, 0.05 % Tween, 50 µM EDTA, 50 µM 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 Na2HPO4/NaH2PO4, 1 M NaCl, pH 7.2
6. Buffer M: 50 mM MES, 150 mM NaCl, pH 6.5

Documents / Resources

	BRUKER heliX plus Thiol Coupling Kit 1 [pdf] User Manual HK-MAL-1, heliX plus Thiol Coupling Kit 1, heliX plus, Thiol Coupling Kit 1 , Coupling Kit 1, Kit 1

References

- User Manual

BRUKER, Coupling Kit 1, heliX Plus, heliX plus Thiol Coupling Kit 1, HK-MAL-1, Kit 1, Thiol Coupling Kit 1

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