USER MANUAL
CK-NH2-1-A96

dynamic BIOSENSORS

Amine Coupling Kit 1 for Proteins (> 5 kDa)

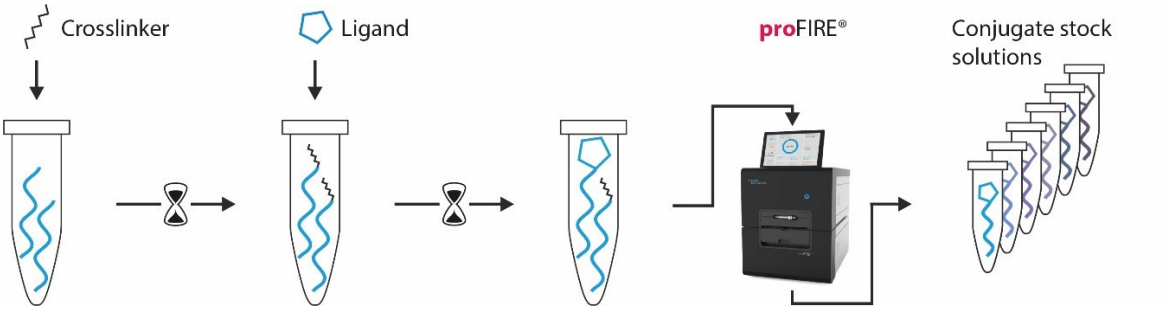
Functionalization of 96mer nanolevers via amines (-NH_2)

Key Features

- Coupling of biomolecules with primary amines (e.g. NH_2-terminus, lysines) to modified nanolevers in a reaction tube
- Convenient standard chemistry
- Applicable for proteins (and peptides) (MW > 5 kDa)
- Longer nanolevers for larger complexes
- Compatible with all **switch**SENSE® *Multi-purpose biochips* carrying sequence A96
- Suitable for parallel measurements via DNA encoded addressing
- Coupling of multiple ligands can be performed simultaneously
- Yields >95 % pure ligand-DNA conjugate with controlled quality of your product
- Includes reagents for five individual conjugation reactions (approx. 10-30 regenerations each; up to 200)
- Compatible with automated standard regeneration process
- **pro**FIRE® purification for pure ligand-DNA conjugates

Workflow Overview

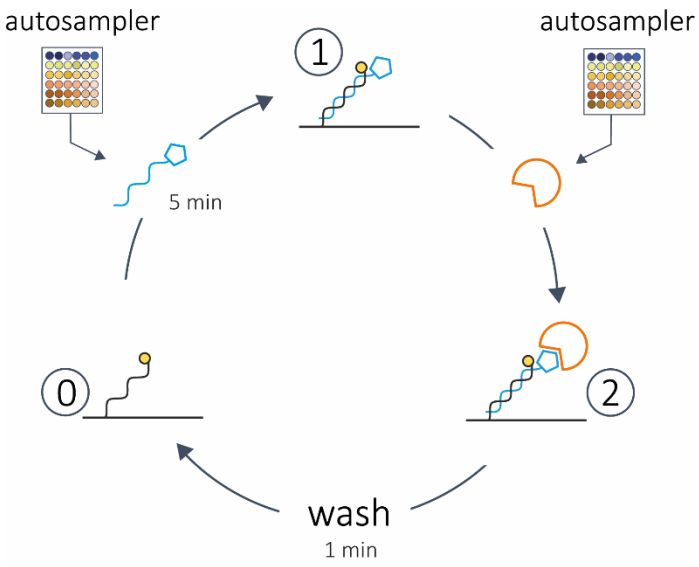
3-Step Conjugation Workflow (in-vitro)



1. DNA Modification	2. Ligand Conjugation	3. Purification	4. Ready-to-use
"complementary nanolevers" (cNL) are activated with amine reactive groups.	After incubation the excess linker is removed by a spin column. The protein/peptide (ligand) is added to the functionalized cNL and incubated for at least 1 h.	The ligand-cNL conjugate is purified using the proFIRE® system. After buffer exchange the conjugates are aliquoted and stored.	The ligand-cNL conjugate stock solutions are ready for insertion in the DRX autosampler.

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

Measurement Workflow with ligand-cNL conjugates



⓪→①

Functionalization of the switchSENSE® biochip with ligands by automatic hybridization of ligand-cNL conjugate.

①→②

Interaction measurement while flowing analyte solution (association) or buffer solution(dissociation) over the chip.

②→⓪

The ligand-cNL analyte complex is washed away from the surface by DNA denaturation under basic pH conditions, which ensures a complete removal of the analyte.

Product Description

Order Number **CK-NH2-1-A96** (nanolever sequence A96)

TABLE 1 | Contents and Storage Information

Material	Cap	Amount	Storage	Comment
cNL-A96-01	blue	5 x	-20°C	
cNL-B96 (1 µM)	yellow	500 µL	-20°C	
Buffer C (50 mM Na_2HPO_4/NaH_2PO_4 pH 8.0, 150 mM NaCl)	trans-parent	5 x 1.8 mL	-20°C	
Buffer A (50 mM Na_2HPO_4/NaH_2PO_4 pH 7.2, 150 mM NaCl)	trans-parent	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	green	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 (10 kDa MWCO)		5 x	r.t.	
Centrifugation collection tube		10 x	r.t.	

For *in vitro* use only.

Please check date of expiry on the kit. 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	Capable between 1,000 x g and 13,000 x g
Vortexer	
1.5 mL reaction tubes	
UV-Vis spectroscopy (e.g. Nanodrop)	Concentration determination of the conjugate

All necessary solutions and buffers are included in the kit.

Important Notes

- Do not use any buffer containing primary amines (i.e. TRIS, glycine) during conjugation process.
- Dithiothreitol (DTT) can be used up to 1 mM during the conjugation process.
  Do not use 2-Mercaptoethanol or other thiol-based reducing agents during 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 get highest reaction yields, the ligand should be dissolved in Buffer C. Buffer exchange is recommended prior to conjugation process[1].
- Before you begin, briefly centrifuge all tubes with blue, green, yellow 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 purification process shall be taken. A few peptides may not give a proper purification using the provided chromatographic column. For more information please email **support@dynamic-biosensors.com**.
- If the pI of the protein is < 6, please make sure to use an adequate buffer.
  For more information, please email **support@dynamic-biosensors.com**.

[1] See page 10 for order no.

3-Step Conjugation of a Biomolecule to a Nanolever in a Reaction Tube

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

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

I Nanolever Modification

1. Equilibrate **two** purification spin columns for one coupling reaction:
   a. Remove column's bottom closure and loosen cap (do not remove cap).
   b. Place 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** on top of column´s resin bed. Centrifuge at 1,500 × g for 1 minute to remove buffer.
   e. Repeat step d once, discard buffer from the reaction tube. The Purification spin column should be in a dry state now.

2. Dissolve cNL-A96-01 (blue cap) in **40 µL Buffer A** prior to use and vortex until solids are completely dissolved and spin down shortly.

3. Dissolve the crosslinker (green cap) by adding **100 µL** ddH_2O and vortex until solids are completely dissolved and spin down shortly. **IMPORTANT**: Always use fresh compounds.

4. Add **10 µL** of the freshly prepared linker solution to one nanolever aliquot (cNL-A96-01, blue cap). Discard the remaining linker solution from step 3.

5. Vortex the reactants for 10 sec, spin down and incubate them for **5 minutes** at room temperature.
   IMPORTANT: Do not exceed incubation time as the reaction yield will decrease.

6. Sample loading
   a. Place columns from step 1 in new 1.5 mL reaction tubes.
   b. Remove cap of spin column number 1 and apply the sample from step 5 to the top of the resin bed.
   c. Centrifuge at 1,500 x g for 2 min to collect the sample (flow-through). Discard Purification spin column after use.
   d. Remove cap of spin column number 2 and apply the sample from step c on top of the resin bed.
   e. Centrifuge at 1,500 x g for 2 min to collect the sample (flow-through). Discard Purification spin column after use.

II Ligand Conjugation

7. Add approx. **100 µg** (up to 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: Be sure that 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 proFIRE® User Manual.

9. Perform a purification using the proFIRE®. Please make sure that the sample volume is 160 µL.
   - If the volume is less than 160 µL, add Buffer C.
   - If it exceeds 160 µL, please perform two subsequent runs.

10. Use the Data Viewer software of the proFIRE® to identify which fractions contains pure conjugate. On page 8 (Additional Information section: proFIRE® Purification of a Ligand-cNL Conjugate) an example chromatogram is shown.

11. Take the recommended fractions out of the fraction collector.

IV Buffer Exchange

12. Add **500 µL** of the first fraction containing the ligand-DNA conjugate from the proFIRE® to the centrifugal filter unit.
    Centrifuge at 13,000 x g (up to 14,000 x g) for **5 minutes** and discard flow-through.

    b. Add the remaining fractions in 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 **5 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.dynamic-biosensors.com/switchsense**).

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

    e. To recover the ligand-DNA 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.

V Optional: Concentration

13. Check ligand-DNA conjugate concentration after buffer exchange by using absorbance at 260 nm and the following equation:

    c (ligand-DNA conjugate)= $A_{260\ nm}$/(1,044,000 L mol^{-1} cm^{-1} * d)

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

VI Aliquots and Storage

14. 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.

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

    IMPORTANT:
    Please add prior to an interaction measurement the appropriate reference cNL (e.g. cNL-B96) to the conjugate solution.

Additional Information

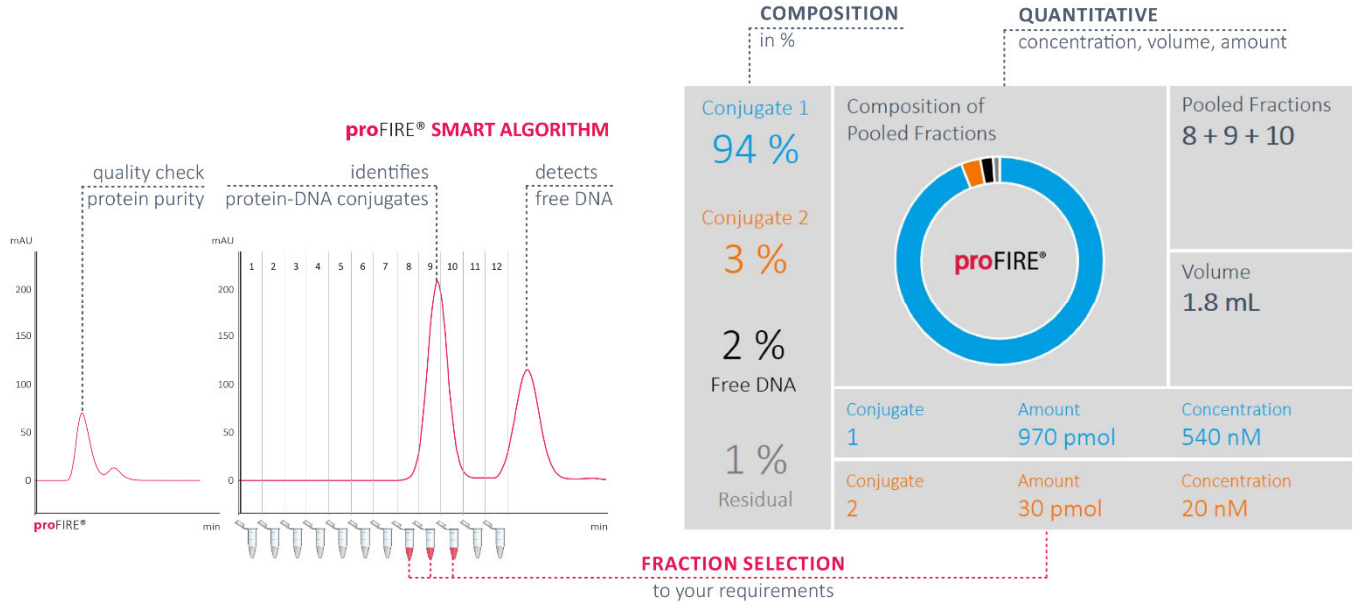
I proFIRE® Purification of a Ligand-cNL Conjugate

1. To perform kinetic analysis it has to be ensured that there is no free DNA on the chip and only 1:1 conjugates are present. Therefore crude ligand-cNL-A96 conjugates have to be purified by ion exchange chromatography. 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 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].

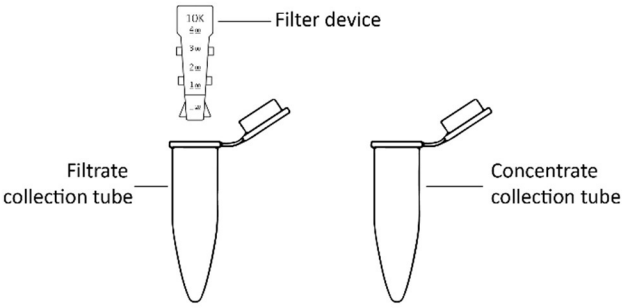
3. Collect the ligand-cNL conjugate fraction (here: 8-10), concentrate the conjugate and exchange buffer with your buffer of choice using a Centrifugal filter unit, as described in section II (Additional information, page 9).

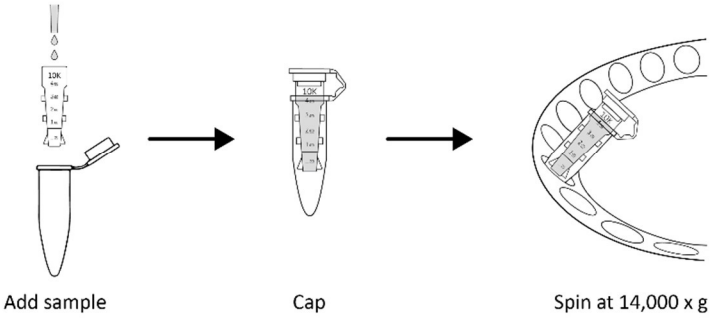


proFIRE® chromatogram of a ligand-cNL-A96 conjugate purification.
Used buffers: Buffer A: 50 mM Na_2HPO_4/NaH_2PO_4 pH 7.2, 150 mM NaCl; Buffer B: 50 mM Na_2HPO_4/NaH_2PO_4 pH 7.2, 1 M NaCl.
Column: DBS-Chromatographic column. *Flow:* 1 mL/min.
Used program: DNA length **96**, *Type* **1**.

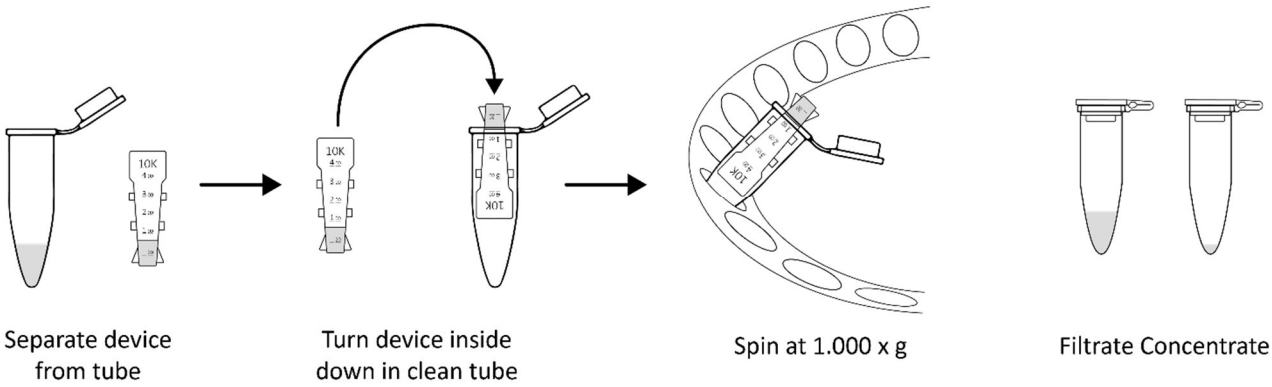
[1] See page 10 for order no.

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 the 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
Amine coupling kit 1 for proteins (>5 kDa); cNL-B96 and NHS modifier, sufficient for 5 conjugation series	CK-NH2-1-B96
Amine coupling kit 2 for proteins (>5 kDa); cNL-B96 and NHS modifier, sufficient for 5 conjugation series (spin column purification)	CK-NH2-2-B96
Amine coupling kit 7 for proteins (>5 kDa) with a low pI (< 6); cNL-B96 and NHS modifier, sufficient for 5 conjugation series	CK-NH2-7-B96
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
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
1x Buffer C pH 8.0 (12 mL of: 50 mM Na2HPO4/NaH2PO4, 150 mM NaCl)	BU-C-150-1

My Notes

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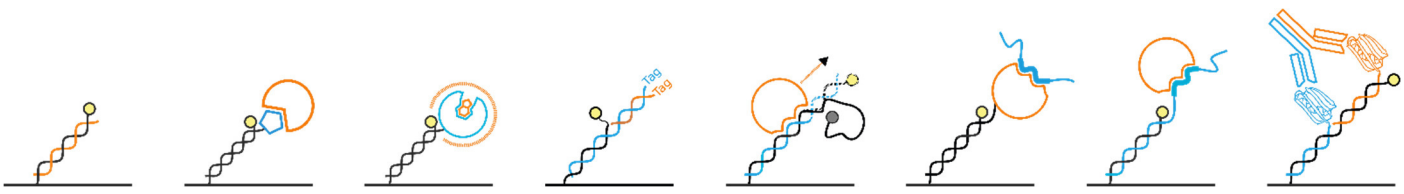

switchSENSE® is a proprietary measurement technology by Dynamic Biosensors GmbH.
Instruments and biochips are engineered and manufactured in Germany.