USER MANUAL
CK-NH2-2-B48

dynamic BIOSENSORS

Amine coupling kit 2 for proteins (> 7 kDa) (Spin column purification)

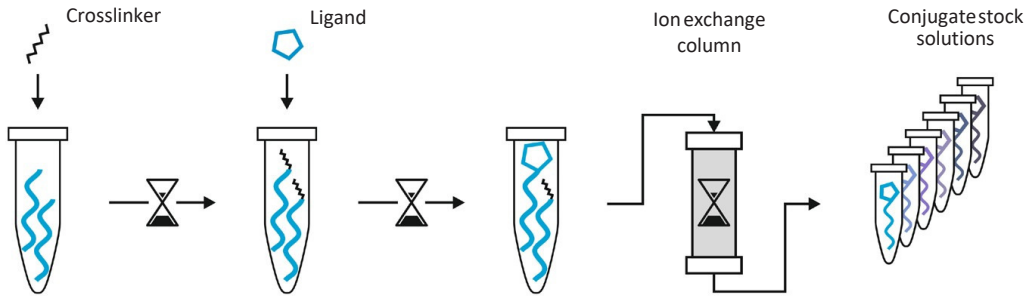
Functionalization of 48mer nanolevers via amines (-NH_2)

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 > 7 kDa)
- Compatible with all **switch**SENSE® *Multi-purpose biochips* carrying sequence B48
- 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. 10-50 regenerations each; up to 500)
- Compatible with automated standard regeneration process

Workflow Overview

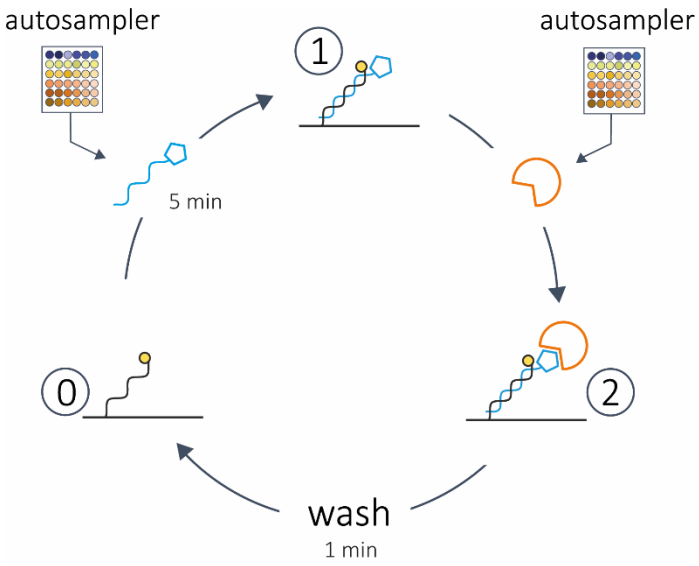
3-Step Conjugation Workflow (in-vitro)



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 provided anion exchange spin columns. 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 **switch**SENSE® 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-NH2-2-B48** (nanolever sequence B48)

TABLE 1 | Contents and storage information

Material	Cap	Amount	Storage	Comments
cNL-B48-01	blue	5 x	-20°C	
cNL-A48 (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 E48 (50mM Na_2HPO_4/NaH_2PO_4 pH 7.2, 618 mM NaCl)	trans-parent	1.5 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.	
Anion exchange (AEX) spin column		5 x	r.t.	
Collection tube for AEX 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. 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 8 for order number).

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 to ensure that all material is at the bottom of the tubes.
- For molecules with a molecular weight around or lower than 7 kDa, special care during purification process shall be taken. A few peptides may not give a proper purification using the provided IEX column. For more information please email **support@dynamic-biosensors.com**.
- If the pI of the protein is < 6, it might be necessary to use CK-NH2-7-B48[1].
  For more information, please email **support@dynamic-biosensors.com**.

[1] See page 8 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-B48-01 (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-B48-01, 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 Purification & Concentration

9. Perform a purification reaction using anion exchange spin columns.
   a. To equilibrate the spin column add 400 µL Buffer A and put the spin column in the provided collection tube. Centrifuge at 2,000 x g for 5 minutes and discard the flow-through.
      Note: To achieve even liquid flow-through the membrane using a fixed-angle rotor, align the printed letter (Q) toward the center of the rotor for all chromatography steps.
   b. Add the complete sample to the spin column, incubate for 1 minute and centrifuge for 2 minutes at 2,000 x g. Discard the flow-through.
   c. Place the column in a new collection tube and add 100 µL of the elution Buffer E48 to the spin column, incubate for 5 minutes and centrifuge for 2 minutes at 2,000 x g. Repeat the elution step and combine the two flow-throughs.

10. Concentration and buffer exchange.
    a. Add **200 µL of PE40** (or TE40, HE40) buffer to the eluted and combined sample from step 9. 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**).
    b. Apply the sample from step a. to the centrifugal filter unit and centrifuge at 13,000 x g (up to 14,000 x g) for **10 minutes** and discard flow-through (Please check on page 7: 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 **15 minutes** and discard flow-through again.
    d. To recover the 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.

11. Determine conjugate concentration by using absorbance at 260 nm and the following equation:
    c (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).

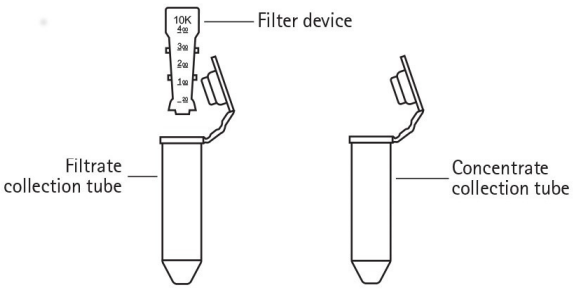
12. Adjust the concentration to **200nM - 1µM** with **PE40** (or TE40, HE40) buffer (including up to 10 % glycerol if needed) and prepare **20 µL** aliquots.

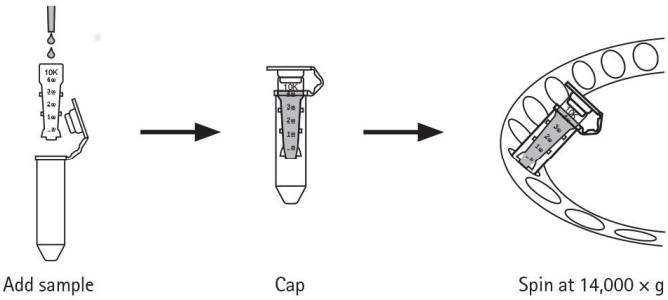
13. Store between 8 °C and -86 °C as desired.

14. Prior to an interaction measurement: Please add the appropriate reference cNL (e.g. cNL-A48) to the conjugate solution.

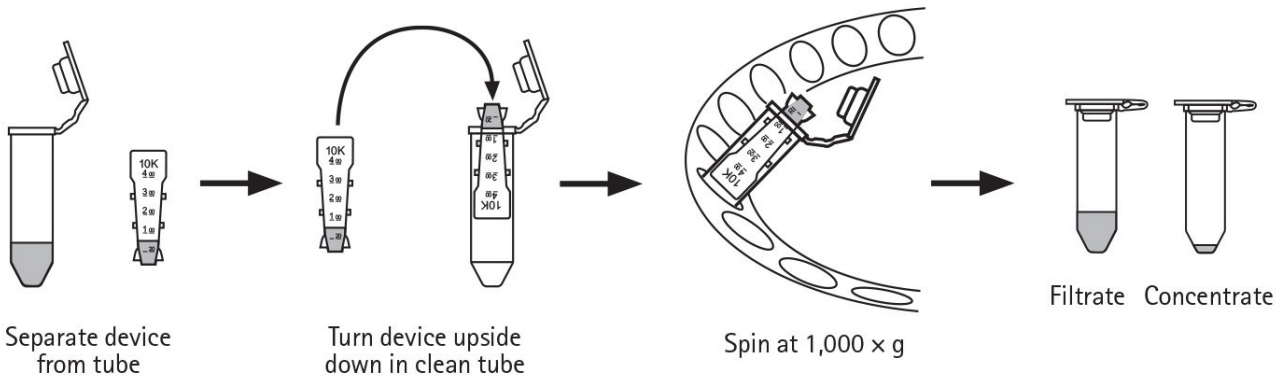
Additional Information

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-B48 and NHS modifier, sufficient for 5 conjugation series	CK-NH2-1-B48
Amine coupling kit 2 for proteins (>7 kDa); cNL-B48 and NHS modifier, sufficient for 5 conjugation series (spin column purification)	CK-NH2-2-B48
Amine coupling kit 7 for proteins (>5 kDa) with a low pI (< 6); cNL-B48 and NHS modifier, sufficient for 5 conjugation series	CK-NH2-7-B48
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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Contact

Dynamic Biosensors GmbH
Perchtinger Str. 8/10
81379 Munich
Germany

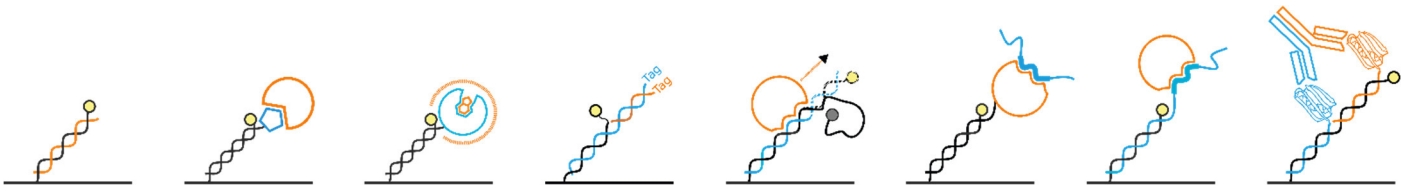
Phone: +49 89 89 74 544 0

Dynamic Biosensors Inc.
300 Trade Center, Suite 1400
Woburn, MA 01801
USA

Phone: +1 781 404 6126

Order Information Phone: +49 89 89 74 544 0
Email: order@dynamic-biosensors.com

Technical Support Phone: +49 89 89 74 544 66
Email: support@dynamic-biosensors.com



switchSENSE® is a proprietary measurement technology by Dynamic Biosensors GmbH.
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