USER MANUAL
PF-NH2-3

dynamic BIOSENSORS

proFIRE® Amine Coupling Kit 3 for Proteins (> 5 kDa)

Functionalization of DBCO-DNA* via amines (-NH_2)

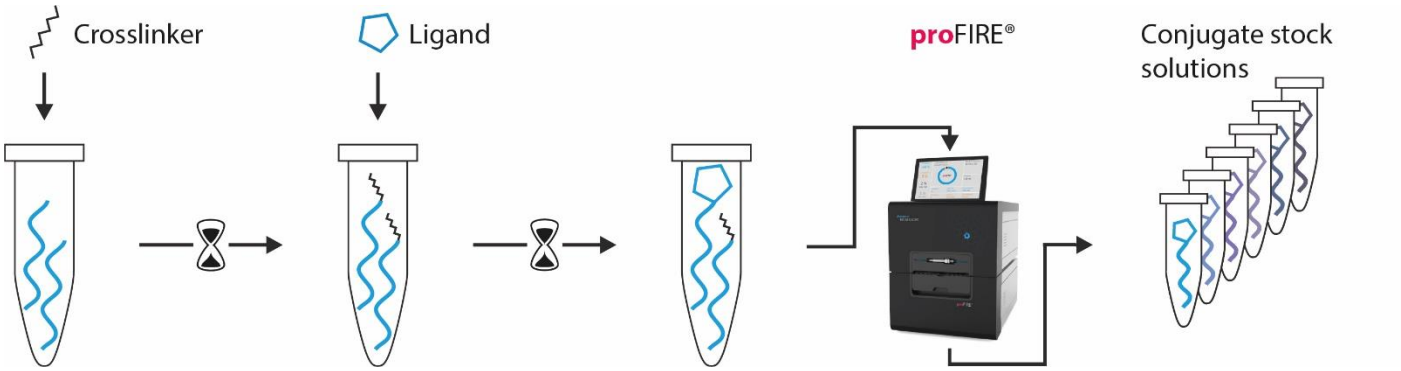
Key Features

- Coupling of biomolecules with primary amines (e.g. NH_2-terminus, lysines) to DBCO-modified DNA* in a reaction tube
- Convenient standard chemistry
- Applicable for proteins (and peptides) (MW > 5 kDa)
- Coupling of multiple proteins can be performed simultaneously
- Yields >95 % pure protein-DNA conjugate with controlled quality of your product
- With any DNA sequence and length up to 150 bases feasible

*Oligos are not included in the kit

Workflow Overview

3-Step Conjugation Workflow (in-vitro)



1. DNA Modification	2. Protein Conjugation	3. Purification	4. Ready-to-use fractions
The DNA is activated with amine reactive groups.	After incubation the excess linker is removed by a spin column. The protein/peptide is added to the functionalized DNA and incubated for at least 1 h.	The protein-DNA conjugate is purified using the **pro**FIRE® system.	The fractions with protein-DNA conjugate are ready for further processing.

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

Product Description

Order Number **PF-NH2-3**

TABLE 1 | Contents and Storage Information

Material	Cap	Amount	Storage	Comment
Conjugation Buffer	trans-parent	5 x 1.8 mL	-20°C	
Dilution Buffer	trans-parent	1.8 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. 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 9 for order number).

Additional Materials Required

TABLE 2 | Additional Materials.

Material	Comment
DNA	We recommend to use 3 - 4 nmol DNA (modified with DBCO, HPLC grade) for one reaction
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.
- 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 Conjugation Buffer. Buffer exchange is recommended prior to conjugation process[1].
- Before you begin, briefly centrifuge all tubes with 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 purification process shall be taken. A few peptides may not give a proper purification using the provided **pro**FIRE® column. For more information please email **support@dynamic-biosensors.com**.
- If the pI of the protein is < 6, it might be necessary to use a lower pH buffer.
  For more information, please email **support@dynamic-biosensors.com**.

[1] See page 9 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.

Before you begin: Allow the crosslinker to reach room temperature before use.

I Nanolever Modification

1. Dissolve the DNA in **40 µL Dilution Buffer** prior to use and vortex until solids are completely dissolved and spin down shortly.

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

3. Add **10 µL** of the freshly prepared linker solution to one DNA aliquot. Discard the remaining linker solution from step 3.

4. Vortex the reactants for 10 sec, spin down and incubate them for **20 minutes** at room temperature.
   IMPORTANT: Do not exceed incubation time as the reaction yield will decrease.

5. In the meantime, equilibrate **two** purification spin columns (red cap) 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 Conjugation Buffer** 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.

6. Sample loading
   a. Place columns from step 5 in new 1.5 mL reaction tubes.
   b. Remove 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 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 Protein Conjugation

7. Add approx. **100 µg** (up to 200 µg) of the protein (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 protein does not contain any primary amines, e.g. TRIS buffers, glycine (please see page 4, Important Notes).

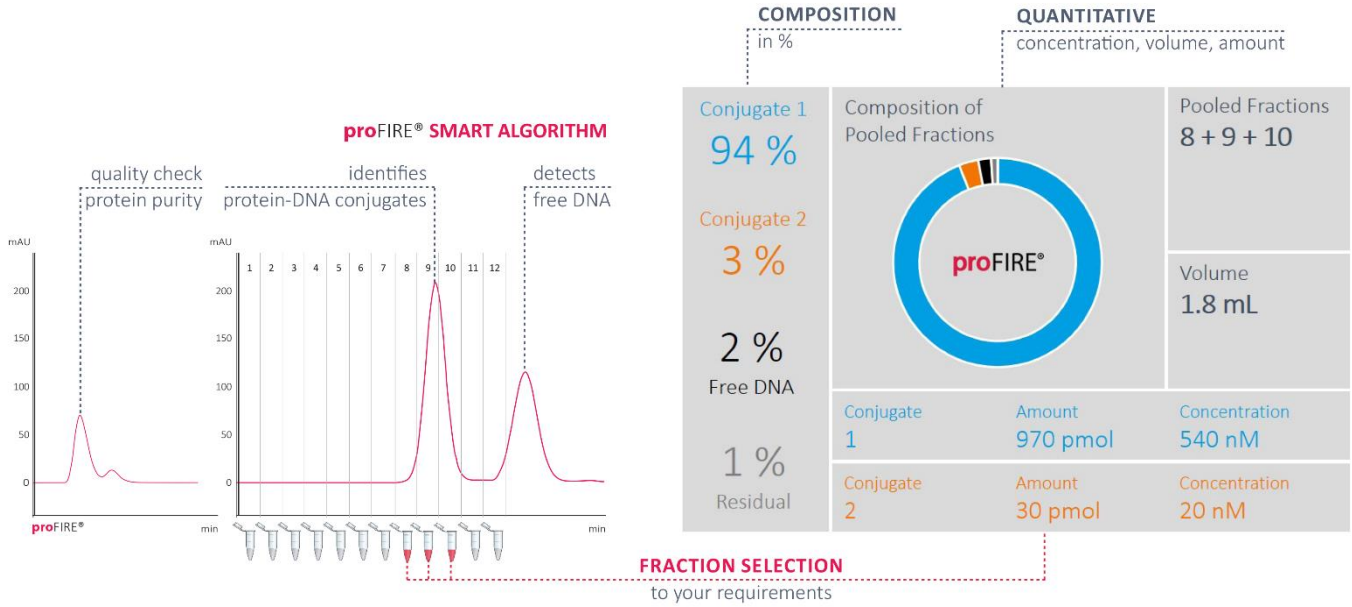
8. Mix the reaction by pipetting up and down and let it react at room temperature for **at least 1 hour**.
   IMPORTANT: Do not vortex. If necessary, the reaction can be carried out at 4 °C with a longer reaction time (e.g. overnight).

III proFIRE® Purification

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

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

10. Use the Data Viewer software of the **pro**FIRE® to identify which fractions contains pure conjugate.
    Example chromatogram:



proFIRE® **chromatogram of a protein-DNA conjugate purification.**
Used buffers: **pro**FIRE® Buffer A; **pro**FIRE® Buffer B.
Column: **pro**FIRE® column. *Flow:* 1 mL/min.
Used program: DNA length *48*. Type: *1*.

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

12. a. Option 1: Store fractions between 8 °C and -86 °C as desired.

    b. Option 2: Proceed with Buffer Exchange and Concentration (see section IV).

IV Optional: Buffer Exchange and Concentration

13. a. Add **500 µL** of the first fraction containing the protein-DNA conjugate from the **pro**FIRE® 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 in the same filter unit and repeat the centrifugation step in order to collect all samples in one tube (Please check on page 8: Additional information for the right use of centrifugal filter unit).

    c. Add **350 µL** of the buffer of choice for buffer exchange and centrifuge at 13,000 x g for **10 minutes**. Discard the flow-through again.

    d. Add **350 µL** of the buffer of choice for buffer exchange and centrifuge at 13,000 x g for **15 minutes**. Discard the flow-through again.

    e. To recover the protein-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.

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

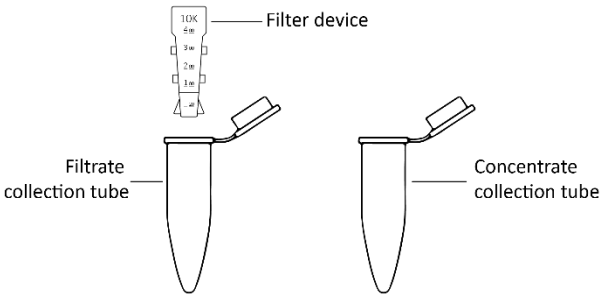
    $c\ (\text{protein-DNA conjugate}) = A_{260\ nm}/(\varepsilon * d)$

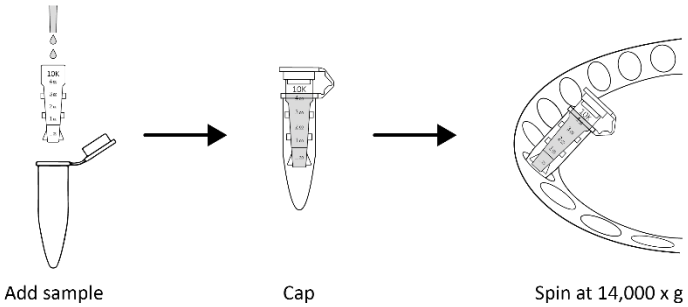
    ε = Extinction Coefficient of the DNA
    d = optical path length
    (usually d = 1 cm, please check photometer manual for further information).

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

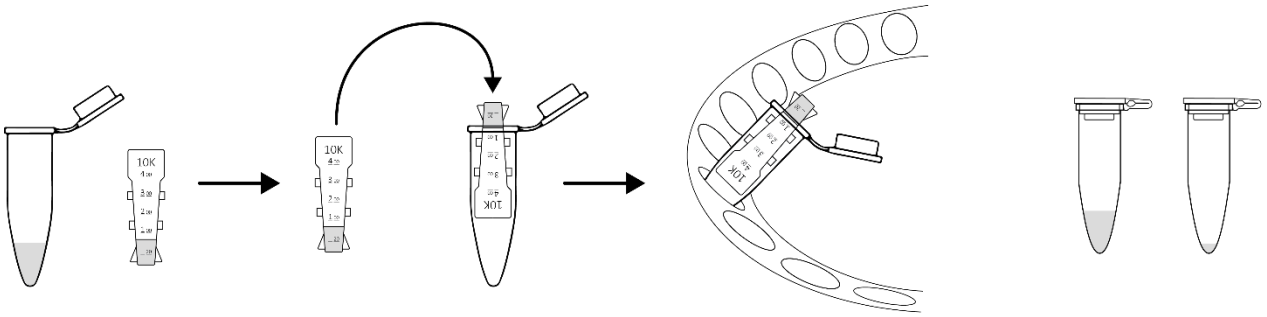
Additional Information

I 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
proFIRE® Antibody Oligo Conjugation Kit; sufficient for 3 conjugation series	PF-AB-1
proFIRE® Amine Coupling Kit 1 for proteins (>5 kDa) with thiol-DNA; sufficient for 5 conjugation series	PF-SH-1
proFIRE® Thiol Coupling Kit 1 for proteins (>5 kDa); sufficient for 5 conjugation series	PF-SH-1
Centrifugal filter unit (3 kDa MWCO), 5 pcs.	CF-003-5
Centrifugal filter unit (10 kDa MWCO), 5 pcs.	CF-010-5
proFIRE® column	PF-CC-1
10x proFIRE® Buffer A (50 mL)	PF-BU-A-10
5x proFIRE® Buffer B (50 mL)	PF-BU-B-5
1x Conjugation Buffer (12 mL)	PF-BU-C-1

My Notes

My Notes

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Get it on Google Play.
Download on the App Store.

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