proFIRE
User Manual

dynamic
BIOSENSORS

Purification column

Description and handling instructions

Dynamic Biosensors GmbH & Inc.

PF-CC-1 v5.1

Product Description

Order Number: PF-CC-1

Table 1. Contents and Storage Information

Column Specifications		Media Specifications	
Column Volume	1 mL	Matrix	Hydrophilic porous polymer beads
Column material	Polypropylene	Particle size	30 µm
Column size length x I.D.	26 x 7.0 mm	Functional group	-R-$N^{+}(CH_3)_3$
Recommended flow rate	1 mL/min	pH range	2 - 12
Maximum flow rate	4 mL/min	Temp. range	4 - 60 °C
Max. pressure	0.3 MPa	Shipping solvent	20 % EtOH aqueous solution

Handling

Storage and Lifetime

Store in 20 % ethanol at 4 - 35 °C.

For longer lifetime, flush weekly the column with water, then with 20 % EtOH solution. Make sure to close the end tightly to avoid drying out.

Equilibration and Elution

Protein-DNA conjugates are electrostatically bound to the matrix of the column with **pro**FIRE® Buffer A as a first mobile phase, then eluted with a salt-concentration gradient method (**pro**FIRE® Buffer B).

Water-soluble organic solvent (maximum of 30 %) can be added in the mobile phase. Before adding such solvent, make sure that the salt will not precipitate.

Cleaning / Troubleshooting

If the column is showing a change in retention time of the free DNA peak or alterations in peak shape and/or pressure increase, follow the washing steps described below.

1. Place buffer tubing A and B in a bottle with water (filtered) and start a “Clean & Sleep” run via Mobile Control software. Leave the column in.
2. Place the buffer **tubing B** in a bottle with **0.1 M to 0.5 M NaOH** (filtered) and leave the buffer tubing A in water (filtered).
3. **Reverse the column** and connect the column outlet to the inlet-tubing coming from the pump. Do not connect the other outlet of the column to the tubing going to the flow cell.
4. Place the opened outlet of the column in a clean bottle to collect the washing solution.
   NOTE: It should be avoided contaminating the system with the washing solution.

5. Tap the “System Overview” symbol, then tap on “Sample injection” in Mobile control software.
   a. Check if the **fraction valve port** is set to **position 13** (waste position)
   b. Check if the **injection valve port** is set to **position 1** (bypass position)
   c. Select the **pump** on the **left bottom side**
   d. Set the pump parameters for channel B: Select channel **B (100%)**, adjust the flow to **1 mL/min**, tap “Apply” and “Run”.
6. Collect the fluid eluting from the column and leave the flow for 10 - 15 minutes.
7. Set the pump parameters for channel A: Select channel **A (100%)**, adjust the flow to **1 mL/min**, tap “Apply” and “Run”.
8. Collect the fluid eluting from the column and leave the flow for 15 minutes.
9. Stop the flow and **reverse the column** in its original intended position with both PEEK-tubings connected to the system.
10. Put both buffer tubing A and B in a bottle with water (filtered) and start two “Clean & Sleep” runs, one after each other via Mobile Control software.
11. Place the tubing A in (filtered and degassed) **pro**FIRE® Buffer A [1] and the tubing B in (filtered and degassed) **pro**FIRE® Buffer B [2] and start a buffer run.
    Type in the **DNA length 1, inject 160 µL** of **pro**FIRE® Buffer A and press **"Start"**.
12. Start a DNA only run with a known DNA oligo you can refer to.

Useful Order Numbers

Table 2. Order Numbers

Product Name	Amount	Order No
10x Buffer A [1]	50 mL (yielding 500 mL)	PF-BU-A-10
5x Buffer B [2]	50 mL (yielding 250 mL)	PF-BU-B-5
proFIRE® Amine Coupling Kit 1 for proteins (>5 kDa);	5 conjugations	PF-NH2-1
proFIRE® Thiol Coupling Kit 1 for proteins (>5 kDa);	5 conjugations	PF-SH-1

Contact

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Instruments and chips are engineered and manufactured in Germany.

[**1**] Buffer A: 50 mM Na_2HPO_4/NaH_2PO_4, 150 mM NaCl, pH 7.2

[**2**] Buffer B: 50 mM Na_2HPO_4/NaH_2PO_4, 1 M NaCl, pH 7.2