heliX+
User Manual

dynamic
BIOSENSORS

Reducing Agent Kit 1 for Proteins and Antibodies

Chemical reduction of disulfide bonds with TCEP

Dynamic Biosensors GmbH & Inc.

RK-PA-1 v2.1

Key Features

- Allows for **reduction of disulfide bonds using TCEP**.
- Convenient standard chemistry.
- Compatible with **proteins and antibodies** (MW > 20 kDa).
- **Site specific reduction** of antibodies.
- Immediate coupling of reduced molecules is possible (*refer to user manual HK-MAL*).
- Reduction of multiple ligands can be performed at the same time.
- Includes reagents for two individual reduction reactions.

Product Description

Order Number: RK-PA-1

Table 1. Contents and Storage Information

Material	Cap	Amount	Storage
TCEP (500 mM)	Red	2 x 20 µL	-20°C
Reducing Buffer [1]	White	2 x 1.8 mL	-20°C
Purification spin column	Red	4 x	2-8°C
2.0 mL reaction tubes for purification spin column		4 x	RT
Centrifugal filter unit (10 kDa MWCO)		2 x	RT
Centrifugation collection tube		4 x	RT

For research use only.

This product has a limited shelf life, please see expiry date on label.

IMPORTANT

Products may be shipped at different temperatures, but storage should adhere to the guidelines outlined in the Table.
The resin slurry in 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	

All necessary solutions and buffers are included in the kit.

3-Step Reduction of a Biomolecule in a Reaction Tube

Please read the entire protocol before starting and **perform all steps without interruption**.
TIP: This protocol can be performed simultaneously for multiple coupling reactions.

I. *Buffer Exchange of Protein/Antibody*

1. Wet the centrifugal filter unit membrane with 100 µL Reducing Buffer.
2. Add approx. **200 µg** (up to 500 µg) of protein/antibody to the filter unit from step 1. If necessary add Reducing Buffer to the filter until a **maximum volume of 450 µL** is reached and centrifuge at **13,000 x g** (up to 14,000 x g) for **5 min** and discard flow-through.
3. Add **350 µL of Reducing Buffer** and centrifuge at 13,000 x g for 5 min and discard flow-through again.
4. Repeat step 3, one more time with a final centrifugation time of 10 min to get a **final volume of approx. 80 µL**.
5. Collect sample either with a 100 µL pipette into a new tube or by placing the filter device upside down in a new centrifugal tube, centrifuge for 2 min at 1000 x g.

II. *Reducing with TCEP*

1. Prepare a 2.5 mM TCEP solution in Reducing Buffer by adding 1 µL of 500 mM TCEP into 199 µL Reducing Buffer. Prepare always directly before use.
2. Add Reducing Buffer to the sample vial for a total volume of 98 µL and add 2 µL of 2.5 mM TCEP solution to obtain a final concentration of 50 µM TCEP. Mix the reaction by pipetting up and down.
3. Incubate the solution at **37°C** at **400 rpm** for **2 h** to ensure complete reduction.

III. *Purification and Concentration*

1. Equilibrate **two** purification spin columns (red cap) for one TCEP wash out:
   a. Remove the column's bottom seal and loosen cap (do not remove cap).
   b. Place the column in a 2.0 mL reaction tube.
   c. Centrifuge at **1,500 × g** for **1 min** to remove the storage solution.
   d. Add **400 µL of Reducing Buffer** to the column´s resin bed. Centrifuge at **1,500 × g** for **1 min** to remove buffer.
   e. Repeat step d and discard the resulting buffer from the reaction tube. The purification spin column should now be in a dry state.

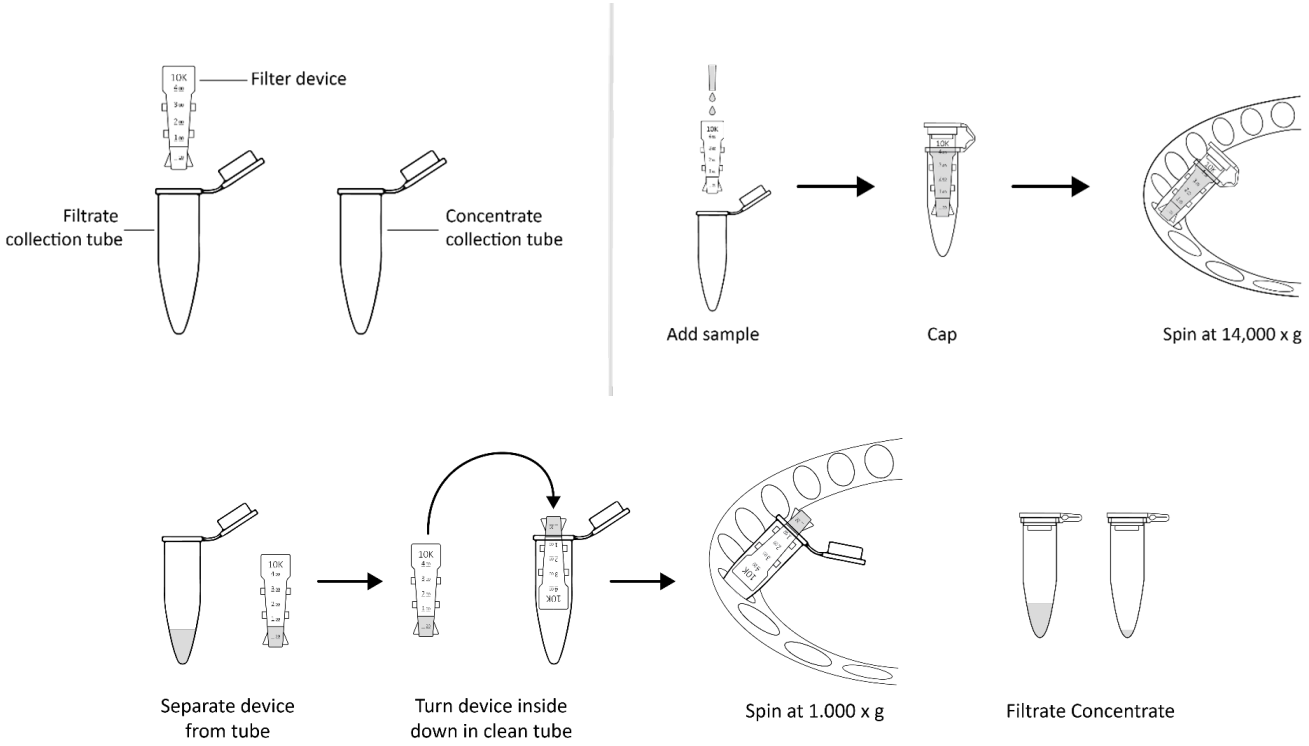
IV. *TCEP wash*

a. Place the columns from the previous step in new 1.5 mL reaction tubes.
b. Remove the cap of **spin column number 1** and pour delicately the sample to the top of the resin bed.
c. Centrifuge at **1,500 x g** for **2 min** to collect the sample (flow-through). Discard the purification spin column after use.
d. Remove the cap of **spin column number 2** and pour the sample from the previous step to the resin bed.
e. Centrifuge at **1,500 x g** for **2 min** to collect the sample (flow-through). Discard the purification spin column after use.
f. Thiol coupling according user manual (*HK-MAL-1 or HK-MAL-2*) of reduced sample can be performed.

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 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 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	Amount	Order No
heliX® Thiol coupling kit 1	5 x	HK-MAL-1
heliX® Thiol coupling kit 2	5 x	HK-MAL-2
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 [2]	50 mL (yielding 500 mL)	BU-P-150-10
5x Buffer B [3]	50 mL (yielding 250 mL)	BU-P-1000-5

My Notes

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Instruments and chips are engineered and manufactured in Germany.

[**1**] Reducing Buffer: 100 mM Na_2HPO_4/NaH_2PO_4, 300 mM NaCl, 5 mM EDTA, pH 7.4

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

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