

## Reducing agent kit 1 for proteins & antibodies

Chemical reduction of disulfide bonds with TCEP

### Key Features

- Reduction of disulfide bonds using TCEP
- Convenient standard chemistry
- Applicable to proteins and antibodies (MW > 20 kDa)
- Immediate coupling of reduced molecules is possible (refer to user manual CK-SH-X-X)
- Site specific reduction of antibodies
- 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	Comment
TCEP (500 mM)	red	2 x 20 µL	-20°C	
Reducing Buffer (100 mM Na <sub>2</sub> HPO <sub>4</sub> /NaH <sub>2</sub> PO <sub>4</sub> pH 7.4, 300 mM NaCl, 5 mM EDTA)	white	2 x 1.8 mL	-20°C	
Purification spin column		4 x	2-8°C	
2.0 mL Reaction tubes for Purification spin column		4 x	r.t.	
Centrifugal filter unit (10 kDa MWCO)		2 x	r.t.	
Centrifugation collection tube		4 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 2 reductions. 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.

## 3-Step Reduction of a Biomolecule 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 Buffer Exchange of Protein/Antibody

1. Wet the centrifugal filter unit membrane with **100 µL** Reducing Buffer (Please check on page 5: Additional information for the right use of centrifugal filter unit).
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 minutes** and discard flow-through.
3. Add **350 µL** of Reducing Buffer and centrifuge at 13,000 x g for **5 minutes** and discard flow-through again. Repeat this step one more time with a centrifugation time of **10 minutes** to get a final volume of approx. **80 µL**.
4. 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 minutes** at 1000 x g.

## II Reducing with TCEP

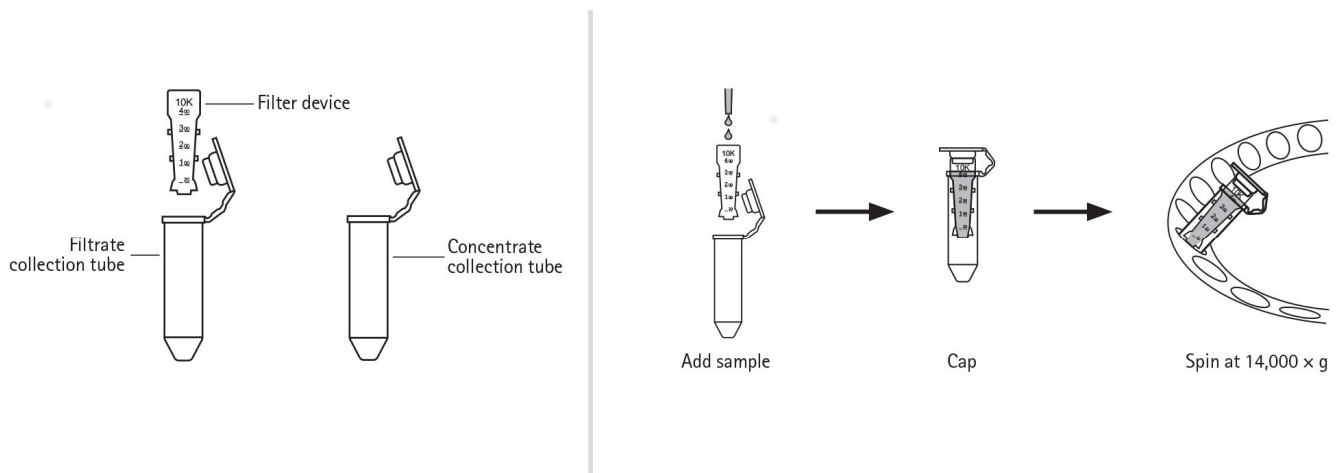
5. Prepare a **2.5 mM** TCEP solution in Reducing Buffer by adding **1  $\mu\text{L}$**  500 mM TCEP to **199  $\mu\text{L}$**  Reducing Buffer. **IMPORTANT:** Prepare always directly before use.
6. Bring the sample volume from step 4 up to **98  $\mu\text{L}$**  with Reducing Buffer and add **2  $\mu\text{L}$**  of **2.5 mM** TCEP solution to obtain a final concentration of 50  $\mu\text{M}$  TCEP. Mix the reaction by pipetting up and down and let it react at **37 °C / 400 RPM** for **2 hours**.

## III Purification & Concentration

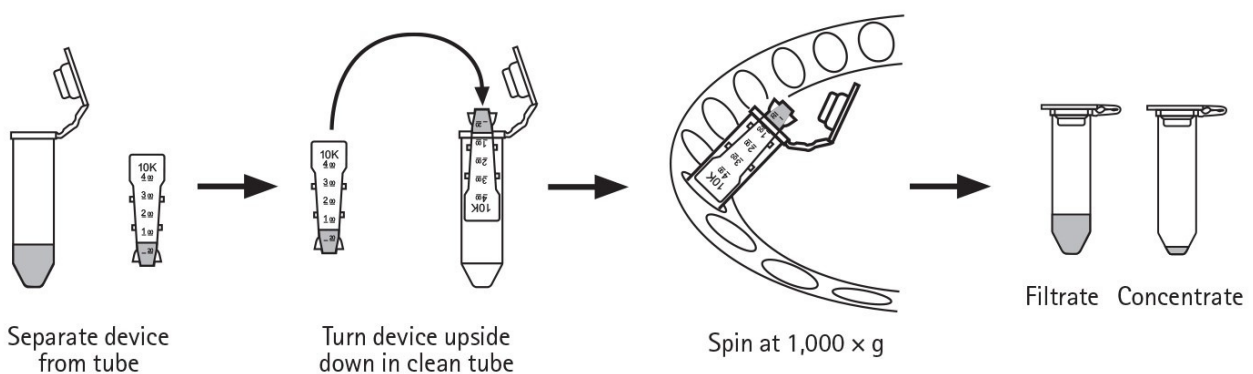
7. Equilibrate **two** Purification spin columns for TCEP wash out:
  - a. Remove column's bottom closure and loosen cap (do not remove cap).
  - b. Place column in a 2.0 mL reaction tube.
  - c. Centrifuge at **1,500  $\times$  g** for **1 minute** to remove storage solution.
  - d. Add **400  $\mu\text{L}$**  of Reducing Buffer on top of the resin bed. Centrifuge at **1,500  $\times$  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.
8. TCEP wash out:
  - a. Place column in a new 1.5 mL reaction tube, remove cap and apply the sample from **step 6** to the top of the compact resin bed.
  - b. Centrifuge at **1,500  $\times$  g** for **2 minutes** to collect the sample (flow-through). Discard purification column after use.
  - c. Apply the sample on top of the resin bed of purification column number 2.
  - d. Centrifuge at **1,500  $\times$  g** for **2 minutes** to collect the sample. Discard purification column after use.
  - e. Thiol coupling according user manual (CK-SH-1-B48, CK-SH-1-B96, CK-SH-2-B48, CK-SH-3-B48) 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 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
Thiol coupling kit 1 for proteins (>5 kDa); cNL-B96 and MAL modifier, sufficient for 5 conjugation series	CK-SH-1-B96
Thiol coupling kit 1 for proteins (>5 kDa); cNL-B48 and MAL modifier, sufficient for 5 conjugation series	CK-SH-1-B48
Thiol coupling kit 2 for proteins (>5 kDa); cNL-B48 and MAL modifier, sufficient for 5 conjugation series (spin column purification)	CK-SH-2-B48
Centrifugal filter unit (3 kDa MWCO), 5 pcs.	CF-003-5
Centrifugal filter unit (10 kDa MWCO), 5 pcs.	CF-010-5

## My Notes

## Contact

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### Order Information

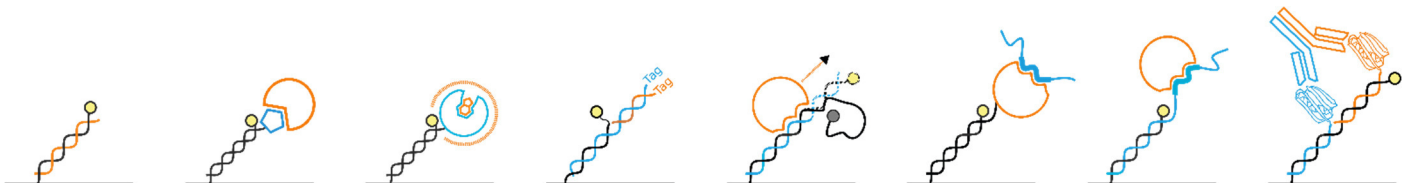
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