Protein Purify&Concentrate Kit

1. Formats

Applicable to the following formats:

Ref No 020002173 – 5 x 3K centrifugal filter devices (0.5 mL)

Ref No 020003173 – 5 x 10K centrifugal filter devices (0.5 mL)

Ref No 020004173 – 5 x 30K centrifugal filter devices (0.5 mL)

Ref No 020005173 – 5 x 50K centrifugal filter devices (0.5 mL)

Ref No 020006173 – 5 x 100K centrifugal filter devices (0.5 mL)

2. Description

Antibodies/proteins often contain low molecular unwanted additives (e.g.: glycine, glycerol, azide, Tris, etc.) that could interfere their labelling with with oligonucleotides (OligoLink Kit antibody) or their conjugation to nanoparticles (LinkOriented GOLD or MAGNETIC Kits). It could also happen that the antibody solution is already purified but it is in a non-adequate buffer for conjugation or its concentration is too low.

Protein Purify&Concentrate kit offers a quick, and easy way of:

i) **Desalting:** removal of low molecular unwanted additives often found in antibodies or protein formulations.

ii) **Concentration:** concentration of dilute antibody or protein solutions.

iii) **Buffer exchange:** removal or exchange of salts, rapidly change of the ionic or pH environment of your antibody or protein solution.

Purification, concentration and desalting of your antibody or protein solution is achieved simultaneously by ultrafiltration of the sample through a regenerated cellulose membrane. The primary basis for separation is molecular size. Centrifugal forces drives solvents and low molecular weight molecules through the membrane into the collecting vial. Molecules larger than the membrane pores will be retained above the membrane inside the sample reservoir (See Figure 1).

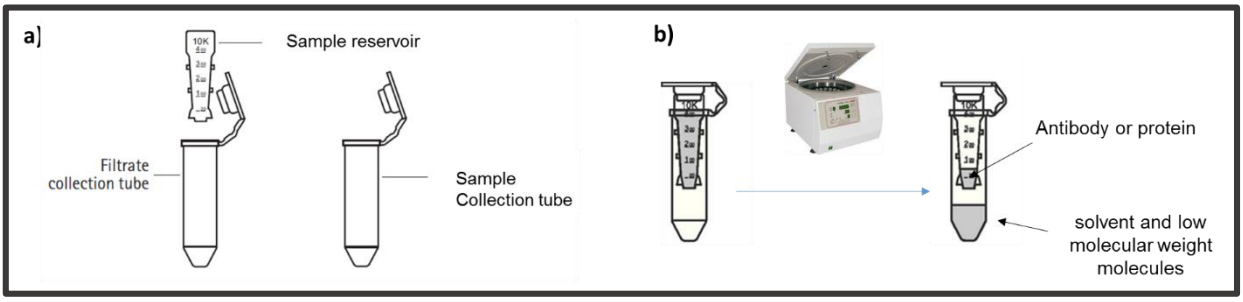

Figure 1. ***a)*** Protein Purify&Concentrate Kit. **b*)*** Assembly of the sample reservoir and the collecting vial.

3. Product Features

- Avoids spinning to dryness
- Typical final concentrate volume of 15-20 µL.
- Provides predictable concentration factor (up to 30 times).
- Inversion of the sample provides consistent antibody/protein recoveries due to minimal protein adsorptive losses to the membrane and the reservoir wall.
- Fast processing ~ 10 to 30 minutes depending on Molecular Weight Cut Off (MWCO) (hands on less than 10 minutes)

4. Kit Content

- 5 sample reservoirs
- 5 filtrate collecting tubes (used to collect filtrate)
- 5 sample collecting tubes (used to recover the concentrated and/or purified sample)

Material/equipment needed but not included on this kit:

- Centrifuge with fixed angle rotor that can accommodate 1.5 mL microcentrifuge tubes.
- Conjugation or Reaction Buffer (i.e. Buffers from all Bioconjugations kits)

NOTE: Protein Purify&Concentrate filter devices are designed for use in centrifuges with fixed-angle rotors. In this way, the filtration rate remains consistently high. In a swinging-bucket rotor, the horizontal angle would force the retentate layer to accumulate over the entire membrane surface, significantly reducing solvent flow.

5. Instructions

Option 1: Concentration of Protein or Antibody solution

1. Select the most appropriate membrane cut-off for your sample. See Section 7. - Selection of the most adequate filtering device)

Note: For maximum recovery select a Molecular weight cut off (MWCO) at least 2 times smaller than the molecular size of the biomolecule of interest.

2. Insert a sample reservoir into one of the provided filtration collection tubes.
3. Add 500 µL of Conjugation or Reaction Buffer to the sample reservoir.
4. Place the capped filter device into the centrifuge rotor, aligning the cap strap toward the center of the rotor; counterbalance with a similar device.

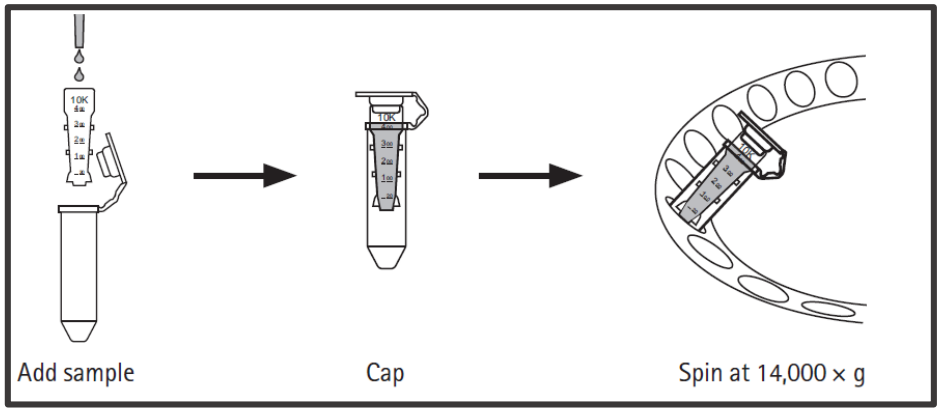

Figure 2. How to place the filter device into the centrifuge rotor.

5. Spin the device for 3 minutes at 14,000 × g to pre-rinse the sample reservoir.

CAUTION: Do not allow the membrane of the filter devices to dry out once wet. If you are not using the device immediately after this step, leave ddH_20 on the membrane until the device is used.

6. Remove the assembled device from the centrifuge and separate the sample reservoir from the filtrate collecting tube.
7. Discard the buffer collected in the filtrate collection tube.
8. Insert the pre-rinsed sample reservoir again into the filtration collection tube.
9. Add up to 500 µL of antibody/protein solution to the sample reservoir.
10. Place the capped filter device into the centrifuge rotor, aligning the cap strap toward the center of the rotor; counterbalance with a similar device.
11. Spin the device for 10 to 30 minutes at 14,000 × g to reduce the buffer volume in the filter.

NOTE: Spin times will vary depending on the MWCO of the selected centrifugal filter device. For typical spin times, see Section 8. - Selection of the spinning time.

12. Remove the assembled device from the centrifuge and separate the sample reservoir from the filtrate collecting tube.
13. To recover the concentrated antibody/protein sample, place the sample reservoir upside down in a sample collecting tube. Place in centrifuge, aligning open cap towards the center of the rotor; counterbalance with a similar device.

14. Spin for 2 minutes at 1,000 × g to transfer the concentrated sample from the sample reservoir to the tube.

Note: For optimal recovery, perform steps 12 and 13 immediately

Caution: Other proteins present in the buffer such as BSA will also be concentrated using this method.

15. The concentrate antibody/protein sample can be stored in the sample colleting tube.

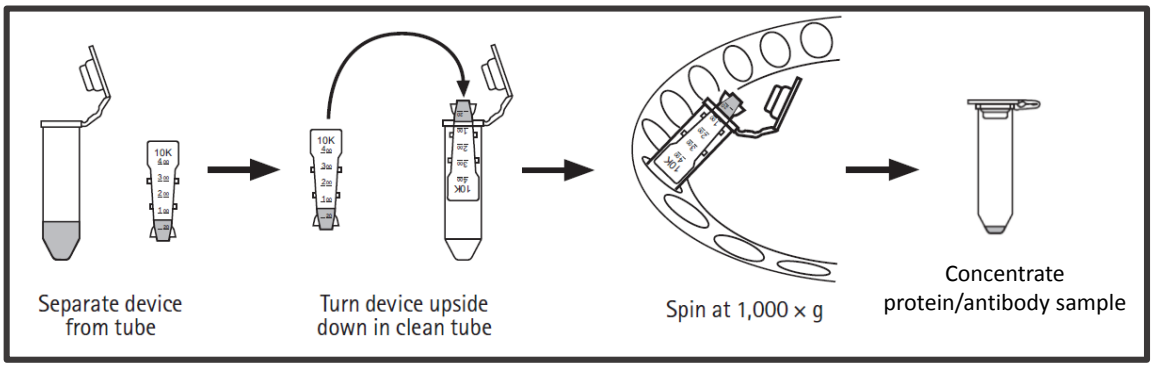

Figure 3. How to recover the concentrate protein/antibody sample.

Option 2: Buffer exchange/purification of antibody solution

1. Add up to 500 µL of antibody/protein solution to the sample reservoir.
2. Place the 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 for 10 to 30 minutes at 14,000 × g to reduce the buffer volume in the filter.

NOTE: Spin times will vary depending on the MWCO of the selected centrifugal filter device. For typical spin times, see Section 8. - Selection of the spinning time.

4. Remove the assembled device from the centrifuge and separate the sample reservoir from the filtrate collecting tube.
5. Discard the filtrate liquid in the Filtrate Collection Tube.
6. Reconstitute the concentrate sample to the original sample volume with any desired buffer (Conjugation Buffer or Reaction Buffer).
7. Insert the sample reservoir into the filtration collection tube.
8. Repeat all these steps (2-7) until the concentration of the contaminating low molecular molecule, salt or buffer has been sufficiently reduced. See example below.

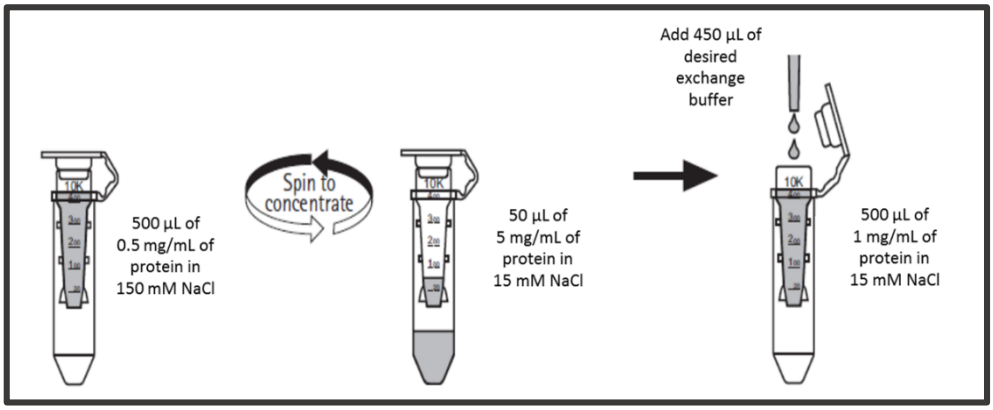

Figure 4. Example of desalting of a protein sample.

Note: Each cycle reduces the concentration of low molecular weight substances (contaminant molecules, salts, etc.). To achieve more complete removal, multiple concentration and reconstitution spins are required.

Caution: The concentration of any unwanted protein in the sample will remain unchanged. To remove unwanted proteins use an adequate purification strategy.

9. To recover antibody solution, place the sample reservoir upside down in a sample collecting tube. Place in centrifuge, aligning open cap towards the center of the rotor; counterbalance with a similar device.
10. Spin for 2 minutes at 1,000 × g to transfer the purified sample from the sample reservoir to the sample tube.

Note: For optimal recovery, perform steps 9 and 10- immediately

11. The purified antibody/protein sample can be stored in the sample colleting tube.

6. Shipping and storage conditions

The kit is shipped and can be stored at room temperature.

7. Selection of the most adequate filtering device

To select the most adequate filtering device, check the molecular weight of the protein of interest, and use the chart below to select a filtering device with an adequate MWCO.

MW Proteins (daltons)	Filter device MWCO
6000 < MW < 20 K	3000
20 K< MW < 60 *K*	10000
60 K < MW < 100 K	30000
100 K < MW < 200 K	50000
200 k < MW	100000

8. Selection of the spinning time

Spin times will vary depending on the MWCO of the selected centrifugal filter device. For typical spin times, see the chart below.

RECOMMENDED G FORCE	*Starting Volume*	≤ 0.5 mL
	Final Volume	15-20 µL
	Fixed-Angle (35º) rotor	14000 g for concentration spin* 1000 g for reverse spin
CONCENTRATION FACTOR	*Final Volume*	15-20 µL with reverse spin
	Concentration Factor	X25-x30
SPINNING TIME	*3000 MWCO*	30 min.
	10000 MWCO	15 min.
	30000 MWCO	10 min.
	50000 MWCO	10 min.
	100000 MWCO	10 min.

* Maximum relative centrifugal force 15,000 × g

9. How to quantify Ab/Protein recovery yields?

- Use an aliquot of the sample before and after following the mentioned concentration/purification protocol and measure their protein content using any commonly used protein quantification assays, e.g., Lowry, Bradford, BCA, and UV spectroscopic protein assays. Use Reaction Buffer of Conjugation Buffer as blank.
- Insert the result of the protein assay (mg/mL) in this equation:

$$\textbf{Recovery (\%)} = \left(\frac{(mg/ml \;\; of \; concentrate \; or \; purified \; sample \times volume \; (mL) \;\; of \; concentrate \; or \; purified \; sample \;)}{(mg/ml \;\; of original \; sample \times volume \; (mL) \;\; of \; original \; sample}\right) \times 100$$

10. Chemical Compatibility

Protein Purify&Concentrate Kit filtering devices are intended for use with biological fluids and aqueous solutions. Compatible with pH 1 to 9. For other solvents, please contact us.

11. Customer Service and Technical Support

For assistance or additional technical information, please contact us:

Telephone: +34 976 369 300

Email: customer_support@nanoimmunotech.es

Product disclaimer

This nitbioconjugation product is to be used for research purposes only. Unless stated in the documentation of on an individual product label, catalog or other information provided to the buyer, IT IS FORBIDDEN TO USE IT for different purposes, including but not limited to them: in vitro diagnostic, use in food, pharmaceutical purposes, medical purposes, or use in cosmetic products, neither for use in humans nor animals, nor for any commercial purposes. Please refer to www.nitbioconjugation.com for the Material Safety Data Sheet of the product.