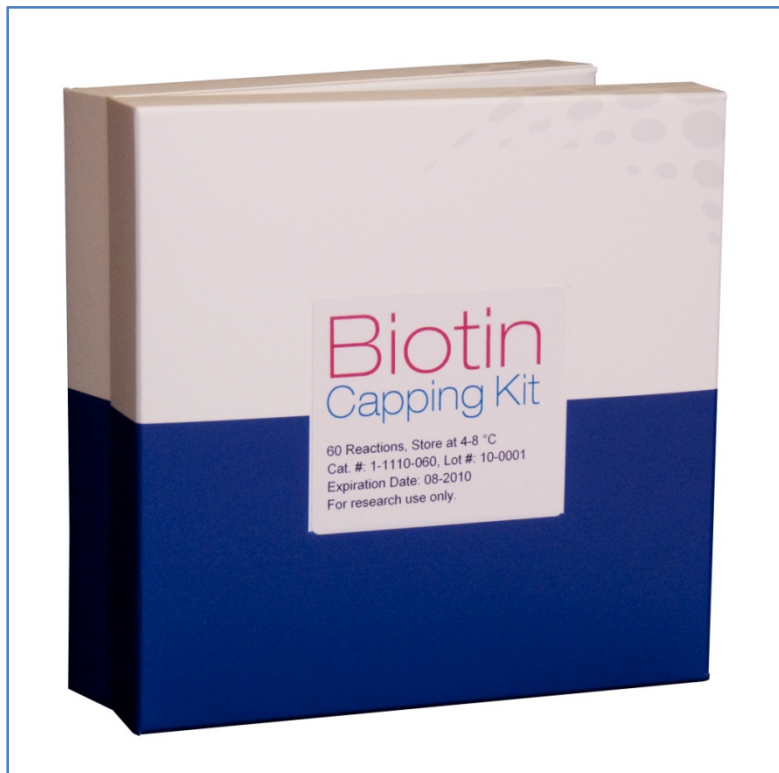


Biotin Capping Kit



When using the Biotin Capping Kit, it is crucial to keep the ratio between biotin and Streptavidin solution constant. The absolute volumes required to cap endogenously biotinylated proteins in different samples depend on the sample used, i.e. on the abundance of endogenously biotinylated proteins. In the absence of the Biotin Capping Kit solutions, endogenously biotinylated proteins, such as some carboxylases in samples of mammalian origin, show up as prominent protein bands. The effect of the capping can be tested by carrying out a capping experiment with different amounts of the kit solutions combined with a capture experiment, and assessment by SDS-PAGE. Capping is complete when no endogenously biotinylated proteins are observable in the capped samples on the gel. Use the amount of Biotin Capping Kit solutions required to abolish these bands for your capture experiments.

We recommend running capture experiments at concentrations of 1-5 mg/ml total protein in the final assay.

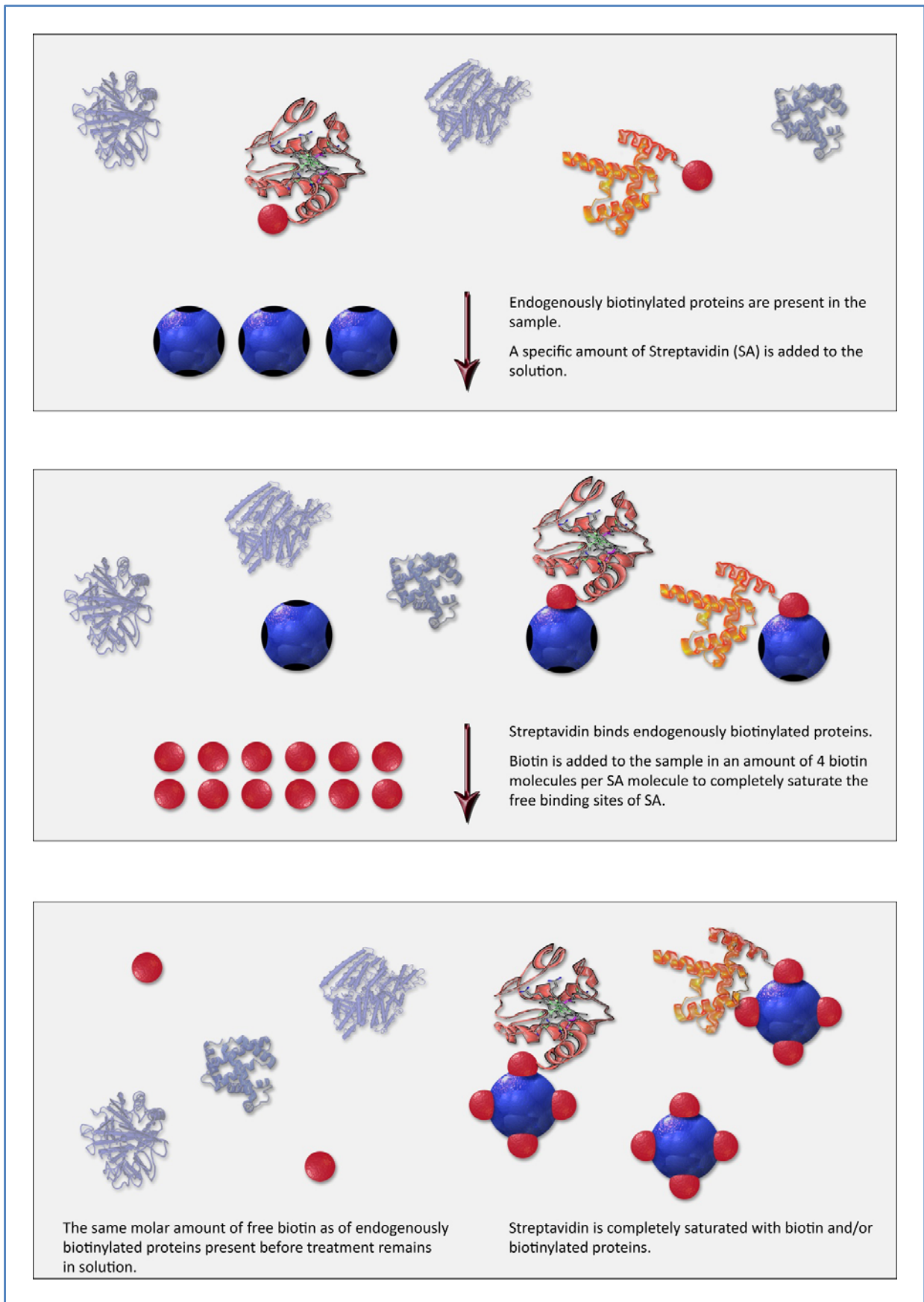


Figure 1: Workflow for application of the Biotin Capping Kit.

Capping reaction

Since the ratio between Streptavidin and biotin is essential for the functionality of the kit, the following precautions and recommendations should be considered.

- Before usage, the Biotin Capping Kit Streptavidin solution and Biotin Capping Kit biotin solution must be at room temperature. It may take up to 45 min for the solutions to thaw and come to room temperature. Only open vials when they have reached room temperature to avoid condensation and, thus, change of concentration of the solutions. Homogenize by inversion and briefly centrifuge.
- Use the same pipette for either solution to avoid volume errors caused by differences in calibration between different pipettes.
- Pay attention on accurate pipetting and ensure complete transfer of both solutions, i.e. Biotin Capping Kit Streptavidin solution and Biotin Capping Kit biotin solution, into the lysate.

1) Prepare capping reaction with the lysate of interest

For example, for *E.coli* lysate we recommend to use 1 µl of Streptavidin solution and 1.3 µl of biotin solution per 56.4 µg of total protein. For HepG2 cell lysate, we recommend 1 µl of Streptavidin and 1.3 µl of biotin solution per 70 µg of total lysate protein. We recommend to test volumes of Streptavidin and biotin solutions for capping assessment on other lysates with 1 µl per 100 µg protein, 1 µl per 50 µg protein, and 1 µl per 25 µg protein. For the conduction of these experiments in practice, use a fixed amount of lysate (we suggest between 200 and 500 µg of total protein and conduct the capping with varying volumes of biotin and Streptavidin solutions, but always with a volume ratio of Streptavidin solution to biotin solution of 1:1.3.

Streptavidin:Biotin [µl]/ [µg] total protein lysate	Recommended for		Initial experiments recommended in other lysates		
	E. coli	HepG2	1:1.3/100	1:1.3/50	1:1.3/25
Total protein amount per assay [µg]	280	350	300	300	300
Streptavidin [µl]	5	5	3	6	12
Biotin [µl]	6.5	6.5	3.9	7.8	15.6

- 2) Add the respective amount of Streptavidin solution to the samples and incubate the lysate without further additives for 20 min at 4 °C under rotation.
- 3) Add the corresponding amount of biotin solution to the samples and incubate the lysate further 20 min at 4 °C under rotation.
- 4) Proceed with the preparation of the Capture Compound™ assay and the competition control as specified in the respective caproKit™ guideline.

For a specific application example please refer to Biotin Capping Kit Application Note. This document can be downloaded at: www.caprotec.com/support/downloads.

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