

Strep-tag[®] AP Detection Kit

(Alkaline Phosphatase)

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1 Use of the protein ladder – general remarks

The included protein ladder comprises 6 different Strep-tag[®]II fusion proteins (each in an amount of 8 μ g) with a molecular weight of 100 kDa, 60 kDa, 45 kDa, 30 kDa, 23.5 kDa, 16 kDa, respectively. It serves as molecular weight standard and positive control in parallel and has to be dissolved with 100 μ l 1x SDS-PAGE sample buffer (final concentration for each marker protein is 80 ng/ μ l) prior to SDS-PAGE. Store the dissolved protein ladder at -20 °C or below.

For 1 - 1.5 mm gels apply 5 μ l of the dissolved protein ladder per lane, for 1.5 - 2.5 mm gels apply 7.5 μ l per lane. Larger amounts of Strep-tag[®] protein ladder (cat. no. 2-1011-100) containing 40 μ g of each marker protein may be purchased separately.

Use standard protocols for gel electrophoresis and electrophoretic transfer of proteins to a membrane. We recommend nitrocellulose as membrane material which provides optimal signal:background ratio.

Covalently biotinylated proteins that occur in expression hosts (e.g. biotin carboxyl carrier protein (BCCP; 21.5 kDa) for *E. coli*) are detected beside the Strep-tag[®] fusion protein. Such specific background bands may serve as internal standard and further positive control during routine use. If it is required to block biotinylated proteins, Biotin Blocking Buffer (Cat. No. 2-0501-002) or Avidin (Cat. No. 2-0204-015) may be added 10 minutes prior to adding Strep-Tactin[®] AP conjugate under 2.2.

2 Protocol

2.1 Blocking

Dilute 10x Buffer SI with distilled water to prepare the required amount of 1x Buffer SI. After protein transfer the membrane is incubated for 30 minutes at room temperature with 1x Buffer SI (0.1-0.5 ml per cm²) on a rocking platform so that the membrane is well covered with liquid.

2.2 Application of Strep-Tactin® AP conjugate

After blocking add 0.25 µl Strep-Tactin® AP conjugate per 1 ml 1x Buffer SI and incubate the blot for additional 30 minutes as described above.

2.3 Washing

Dilute 20x Buffer WD with distilled water to prepare the required amount of 1x Buffer WD.
Wash 1x 10 seconds with 2 ml distilled water per cm² blot surface
Wash 2x 2 minutes with 0.5 ml 1x Buffer WD per cm² blot surface
Wash 1x 2 minutes with 0.5-1.0 ml distilled water per cm² blot surface

2.4 Chromogenic reaction

Prepare the solution for chromogenic reaction (0.25 ml/cm²) freshly before use according to the table below:

Membrane	Buffer ER-AP	+	H ₂ O	+	NBT	+	BCIP
1 cm ²	0.025 ml	+	0.225 ml	+	0.75 µl	+	0.75 µl
2 cm ²	0.050 ml	+	0.450 ml	+	1.50 µl	+	1.50 µl
5 cm ²	0.125 ml	+	1.125 ml	+	3.75 µl	+	3.75 µl
10 cm ²	0.250 ml	+	2.250 ml	+	7.50 µl	+	7.50 µl
20 cm ²	0.5 ml	+	4.5 ml	+	15 µl	+	15 µl
50 cm ²	1.25 ml	+	11.25 ml	+	37.5 µl	+	37.5 µl
100 cm ²	2.5 ml	+	22.5 ml	+	75 µl	+	75 µl
200 cm ²	5 ml	+	45 ml	+	150 µl	+	150 µl
		+		+		+	

Specific protein bands generally appear after 3 to 5 minutes. At low protein amounts per band 15 minutes development or longer may be necessary. Stop enzyme reaction when optimal signal:background staining has been achieved by washing the membrane several times with distilled H₂O. Dry the blot between sheets of absorbent paper (e.g. Whatman 3MM) and store protected from light.

3 References

For up-to-date references see www.iba-go.com