Strep-tag® HRP Detection Kit
(Horse Radish Peroxidase)

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Strep-tag® technology for protein purification and detection is covered by US patent 5,506,121, UK patent 2272698 and French patent 93 13 066; the tetracycline promoter based expression system is covered by US patent 5,849,576 and Strep-Tactin® is covered by US patent 6,103,493. Further patent applications are pending world-wide. Purchase of reagents related to these technologies from IBA provides a license for non-profit and in-house research use only. Expression or purification or other applications of above mentioned technologies for commercial use require a separate license from IBA. A license may be granted by IBA on a case-by-case basis, and is entirely at IBA's discretion. Please contact IBA for further information on licenses for commercial use.

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Content

1 Use of the control protein – general remarks

The control protein (28 kDa; 24 µg) has to be dissolved in 240 µl 1x SDS-PAGE sample buffer (final concentration is 0.1 µg/µl) prior to SDS-PAGE. Store the dissolved control protein at -20 °C or below.

For 1 - 1.5 mm gels apply 5 µl control protein per lane, for 1.5 - 2.5 mm gels apply 7.5 µl per well.

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 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 HRP 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 cm^2) on a rocking platform so that the membrane is well covered with liquid.

2.2 Application of *Strep*-Tactin HRP conjugate

After blocking add 0.25 µl Strep-Tactin HRP 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 cm^2 blot surface
Wash 2x 2 minutes with 0.5 ml 1x Buffer WD per cm^2 blot surface
Wash 1x 2 minutes with 0.5-1.0 ml distilled water per cm^2 blot surface

2.4 Chromogenic reaction

Prepare the solution for chromogenic reaction (0.25 ml/cm^2) freshly before use according to the table below:

Membrane	Buffer ER-HRP	+	H_2O	+	Chromogen	+	HRP substrate
1 cm^2	0.025 ml	+	0.225 ml	+	1.75 µl	+	0.25 µl
2 cm^2	0.050 ml	+	0.450 ml	+	3.5 µl	+	0.50 µl
5 cm^2	0.125 ml	+	1.125 ml	+	8.75 µl	+	1.25 µl
10 cm^2	0.250 ml	+	2.250 ml	+	17.5 µl	+	2.5 µl
20 cm^2	0.5 ml	+	4.5 ml	+	35 µl	+	5 µl
50 cm^2	1.25 ml	+	11.25 ml	+	87.5 µl	+	12.5 µl
100 cm^2	2.5 ml	+	22.5 ml	+	175 µl	+	25 µl
200 cm^2	5 ml	+	45 ml	+	350 µl	+	50 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 H_2O. 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