

Spot-Tag, Spot-Label & Spot-Trap

First universal affinity-tag & Nanobody based system for

- ▶ Immunofluorescence microscopy, i.e. confocal, STED, STORM
- ▶ Immunoprecipitation (IP), Co-IP, Mass spec & Western blot
- ▶ Affinity purification with native and non-native elution

Novel Spot system

ChromoTek's new Spot system consists of a short peptide tag and a highly affine nanobody coupled to fluorophores or matrices.

Benefits

- Universal peptide tag for a broad range of applications
- Optimized for tagged proteins at endogenous levels
- Cutting edge Nanobody technology for highest performance

Universal Spot-Tag®

- 12 amino acid sequence: PDRVRAVSHWSS, Size: 1.4 kDa
- Good expression levels in bacteria, yeast, mammalian cell lines, and insect cells; N-terminally and C-terminally
- Spot-Tag expression vectors (various constructs)

Immunofluorescence & Western blotting: Spot-Label

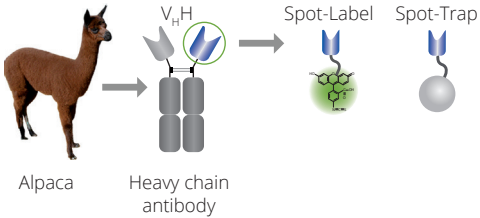
- anti-Spot-Tag Nanobody conjugated to ATTO594
- Minimal linkage error, better tissue penetration
- High binding affinity & excellent performance in IF
- Very sensitive in Western blots

IP, Co-IP & Affinity purification: Spot-Trap®

- anti-Spot-Tag Nanobody coupled to agarose beads
- Single band purification: no heavy and light antibody chains in downstream applications
- Highly affine with a low dissociation constant KD of 6-7 nM for N- respectively C-terminal fusions
- High stability with Tm of 65°C allows harsh washing conditions including 2M NaCl or 2M urea, and a pH range from pH 4 to 10
- Binding capacity ≥1.4 mg/ml resin (for a 30 kDa protein)
- Native elution with Spot-Tag and non-native with pH shift

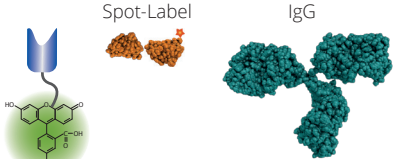
Validated

- Structure and function are fully characterized
- Produced at constant high quality virtually without batch-to-batch variation



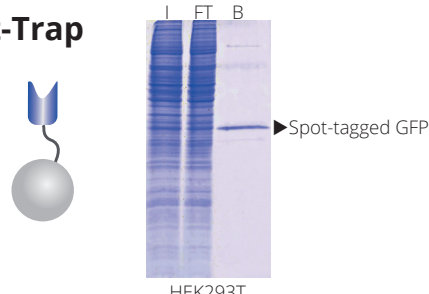
anti-Spot-Tag Nanobody is derived from an alpaca single domain antibody, which is devoid of light chains and bind its antigen via a single variable domain (V_HH). The Nanobody is used for Spot-Label and Spot-Trap.

Spot-Label



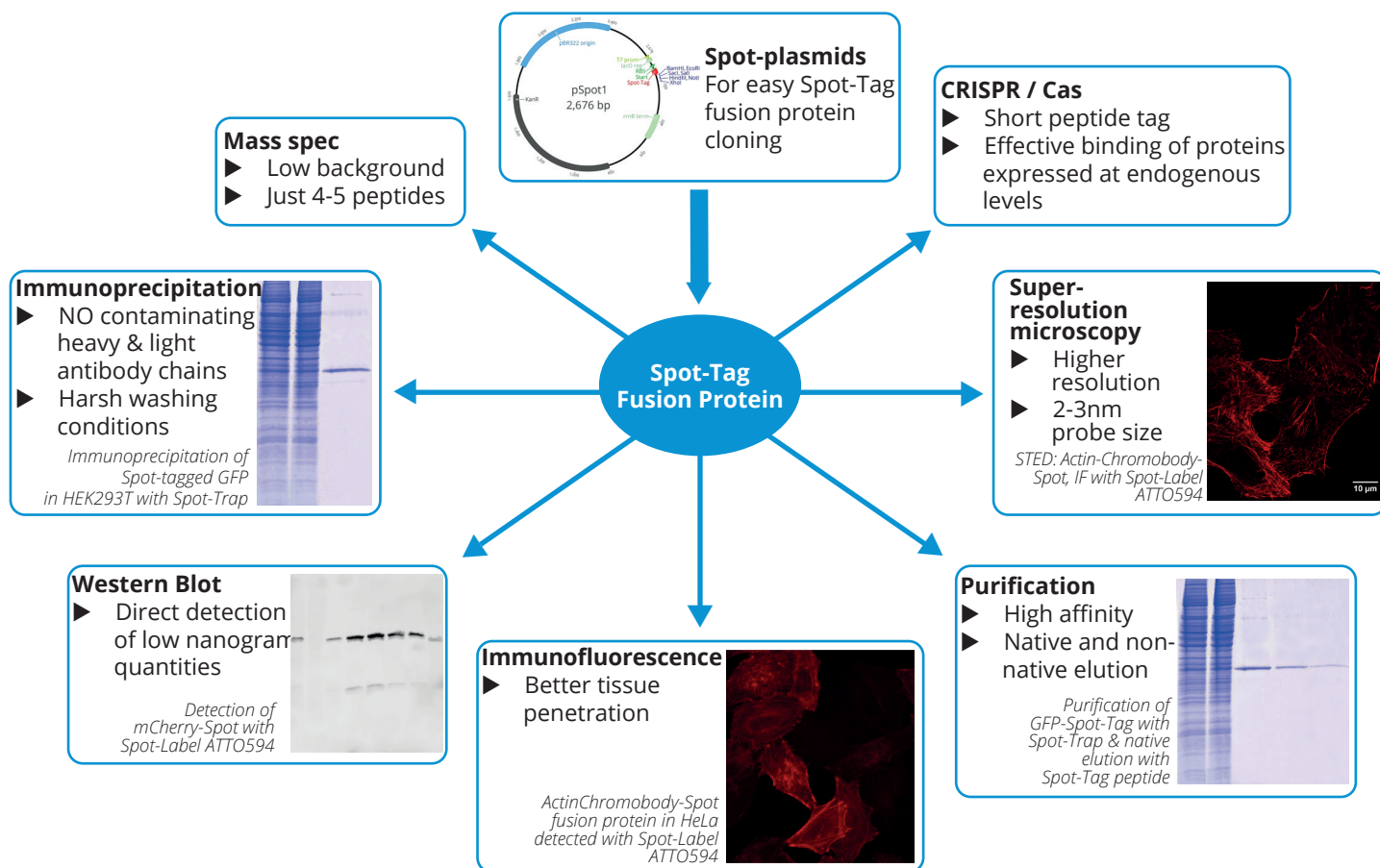
Size comparison scheme of Spot-Label vs. IgG: 30 vs. 150 kDa

Spot-Trap



SDS-PAGE: IP with single band purification of GFP-Spot-Tag fusion protein: Input (I), flow-through (FT) & bound (B) fractions

Spot-Tag: Universal affinity-tag & Nanobody based system



Products (for research only)

Product	Size	Code	Product	Size	Code																																																			
Spot-Trap Agarose	10 rxns (250µl resin)	eta-10	Spot-Tag Peptide, lyophilized	1 mg	ep-1																																																			
	20 rxns (500µl resin)	eta-20		100 rxns (2,5ml resin)	eta-100	Spot-Trap Magnetic Agarose	10 rxns (250µl resin)	etma-10	20 rxns (500µl resin)	etma-20	100 rxns (2,5ml resin)	etma-100	Spot-Label_Atto594 for IF, bivalent	10µl	eba594-10	pSpot1 vector, E. coli, Spot-tag N-term., Kan., high expression	1.25mg	ev-1	50µl	eba594-50	pSpot2 vector, E. coli, Spot-tag C-term., Kan., high expression	1.25mg	ev-2	Spot-Label_Atto488 for WB, monovalent	10µl	ema488-10	pSpot3 vector, E. coli, Spot-tag C-term., Amp., low expression	1.25mg	ev-3	50µl	ema488-50	pSpot4 vector, E. coli, Spot-tag N-term., Amp., low expression	1.25mg	ev-4	Spot-Label, uncoupled for IF, bivalent	50mg	etb-50	pSpot5 vector, S. cerevisiae, Spot-tag N-term., Leu, CEN, low expression	1.25mg	ev-5	250mg	etb-250	pSpot6 vector, S. cerevisiae, Spot-tag C-term., Leu, CEN, low expression	1.25mg	ev-6	Spot-Label, uncoupled for WB & IP, monovalent	50mg	etm-50	pSpot7 vector, S. cerevisiae, Spot-tag N-term., Leu, 2µ, high expression	1.25mg	ev-7	250mg	etm-250	pSpot8 vector, S. cerevisiae, Spot-tag C-term., Leu, 2µ, high expression	1.25mg	ev-8
	100 rxns (2,5ml resin)	eta-100		Spot-Trap Magnetic Agarose	10 rxns (250µl resin)		etma-10	20 rxns (500µl resin)	etma-20	100 rxns (2,5ml resin)	etma-100	Spot-Label_Atto594 for IF, bivalent		10µl	eba594-10	pSpot1 vector, E. coli, Spot-tag N-term., Kan., high expression	1.25mg	ev-1	50µl	eba594-50	pSpot2 vector, E. coli, Spot-tag C-term., Kan., high expression	1.25mg	ev-2		Spot-Label_Atto488 for WB, monovalent	10µl	ema488-10	pSpot3 vector, E. coli, Spot-tag C-term., Amp., low expression	1.25mg	ev-3	50µl	ema488-50	pSpot4 vector, E. coli, Spot-tag N-term., Amp., low expression	1.25mg		ev-4	Spot-Label, uncoupled for IF, bivalent	50mg	etb-50	pSpot5 vector, S. cerevisiae, Spot-tag N-term., Leu, CEN, low expression	1.25mg	ev-5	250mg	etb-250	pSpot6 vector, S. cerevisiae, Spot-tag C-term., Leu, CEN, low expression		1.25mg	ev-6	Spot-Label, uncoupled for WB & IP, monovalent	50mg	etm-50	pSpot7 vector, S. cerevisiae, Spot-tag N-term., Leu, 2µ, high expression	1.25mg	ev-7	250mg	etm-250
Spot-Trap Magnetic Agarose	10 rxns (250µl resin)	etma-10			20 rxns (500µl resin)		etma-20	100 rxns (2,5ml resin)	etma-100	Spot-Label_Atto594 for IF, bivalent	10µl		eba594-10	pSpot1 vector, E. coli, Spot-tag N-term., Kan., high expression	1.25mg	ev-1	50µl	eba594-50	pSpot2 vector, E. coli, Spot-tag C-term., Kan., high expression	1.25mg	ev-2	Spot-Label_Atto488 for WB, monovalent	10µl	ema488-10		pSpot3 vector, E. coli, Spot-tag C-term., Amp., low expression	1.25mg	ev-3	50µl	ema488-50	pSpot4 vector, E. coli, Spot-tag N-term., Amp., low expression	1.25mg	ev-4	Spot-Label, uncoupled for IF, bivalent	50mg	etb-50		pSpot5 vector, S. cerevisiae, Spot-tag N-term., Leu, CEN, low expression	1.25mg	ev-5	250mg	etb-250	pSpot6 vector, S. cerevisiae, Spot-tag C-term., Leu, CEN, low expression	1.25mg	ev-6	Spot-Label, uncoupled for WB & IP, monovalent	50mg	etm-50		pSpot7 vector, S. cerevisiae, Spot-tag N-term., Leu, 2µ, high expression	1.25mg	ev-7	250mg	etm-250	pSpot8 vector, S. cerevisiae, Spot-tag C-term., Leu, 2µ, high expression	1.25mg
	20 rxns (500µl resin)	etma-20			100 rxns (2,5ml resin)	etma-100	Spot-Label_Atto594 for IF, bivalent	10µl	eba594-10		pSpot1 vector, E. coli, Spot-tag N-term., Kan., high expression	1.25mg	ev-1	50µl	eba594-50	pSpot2 vector, E. coli, Spot-tag C-term., Kan., high expression	1.25mg	ev-2	Spot-Label_Atto488 for WB, monovalent	10µl	ema488-10		pSpot3 vector, E. coli, Spot-tag C-term., Amp., low expression	1.25mg	ev-3	50µl	ema488-50	pSpot4 vector, E. coli, Spot-tag N-term., Amp., low expression	1.25mg	ev-4	Spot-Label, uncoupled for IF, bivalent	50mg	etb-50		pSpot5 vector, S. cerevisiae, Spot-tag N-term., Leu, CEN, low expression	1.25mg	ev-5	250mg	etb-250	pSpot6 vector, S. cerevisiae, Spot-tag C-term., Leu, CEN, low expression	1.25mg	ev-6	Spot-Label, uncoupled for WB & IP, monovalent	50mg	etm-50		pSpot7 vector, S. cerevisiae, Spot-tag N-term., Leu, 2µ, high expression	1.25mg	ev-7	250mg	etm-250	pSpot8 vector, S. cerevisiae, Spot-tag C-term., Leu, 2µ, high expression	1.25mg	ev-8		
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