

# Spot-Label® Alexa Fluor® 647

Product code: ebAF647

## Properties

<b>Description</b>	Anti-Spot-Tag® VHH (bivalent) conjugated to Alexa Fluor® 647.
<b>Target / Specificity</b>	Spot-Tag® sequence (PDRVRAVSHWSS). Compatible with N- and C-terminal tagging, internal tagging must be tested case by case.
<b>Species Specificity</b>	N/a
<b>GenBank Accession Number</b>	N/a
<b>GeneID (NCBI)</b>	N/a
<b>Product Type</b>	Primary antibody, bivalent
<b>Format</b>	Recombinant alpaca single domain antibody, bivalent
<b>Source</b>	Alpaca, recombinantly produced
<b>Isotype / Subclass</b>	N/a
<b>Clonality</b>	Monoclonal
<b>Clone</b>	CTK0216
<b>RRID</b>	AB_2889376
<b>Immunogen</b>	Beta-catenin, engineered epitope
<b>Conjugate</b>	Alexa Fluor® 647
<b>Excitation/ Emission</b>	Excitation max: 650 nm, Emission max: 665 nm
<b>Degree of Labeling (DOL)</b>	1 fluorophore per bivalent Nanobody, site-directed conjugation
<b>Purification Method</b>	Recombinantly expressed and purified
<b>Form</b>	Buffered aqueous solution
<b>Concentration</b>	500 µg/mL
<b>Calculated MW</b>	30.3 kDa
<b>Tested Applications</b>	IF
<b>Positive Controls</b>	IF: HeLa cells transfected with pSpot Spot-Tag®-Actin (ev-31)
<b>Cited Applications</b>	
<b>Recommended Dilutions</b>	IF/ICC: 1:800
<b>Storage Buffer</b>	PBS, 5 mM EDTA, preservative: 0.09 % sodium azide Safety datasheet (SDS): sodium azide
<b>Storage Conditions</b>	Aliquot upon receipt and store at -20°C/-4°F. Avoid freeze-thaw cycles. Protect from light.

# Spot-Label<sup>®</sup> Alexa Fluor<sup>®</sup> 647

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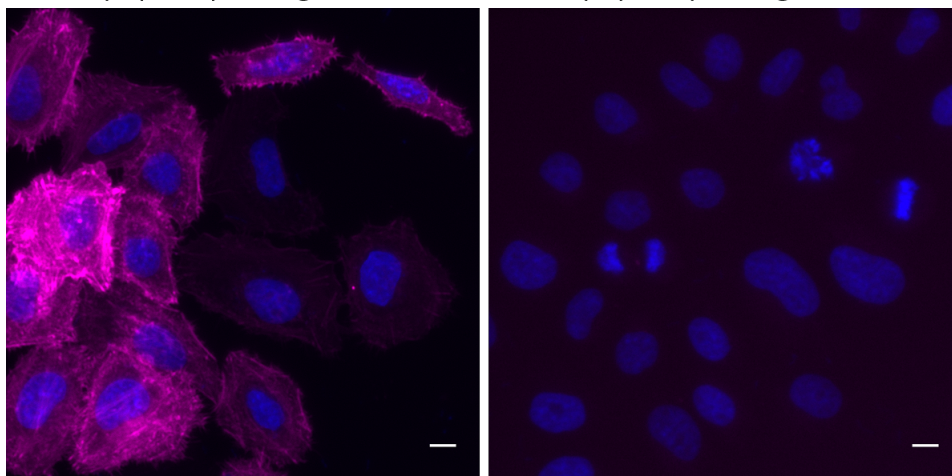
Stability	Stable for 6 months at -20°C/-4°F after shipment.
Shipment	Shipped on ice
Size	10 µL; 50 µL
Synonyms	VHH, Nanobody, alpaca single domain antibody, binding domain of single domain antibody, Nano-antibody

## Selected validation data

### Immunofluorescence

+ pSpot Spot-Tag<sup>®</sup>-Actin

- pSpot Spot-Tag<sup>®</sup>-Actin



*HeLa cells transiently expressing pSpot Spot-Tag<sup>®</sup>-Actin (ev-31) were immunostained with Spot-Label<sup>®</sup> Alexa Fluor<sup>®</sup> 647 (magenta, ebAF647, 1:800) and DAPI (blue). Scale bar, 10 µm.*

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## Immunofluorescence protocol

### Fixation

- Fix cells seeded on coverslips in 3.7% formaldehyde in PBS for 10 min at room temperature.  
*Note: Always prepare a fresh formaldehyde dilution.*
- Wash samples three times with PBS. Do not store fixed cells.

### Permeabilization

- Add 0.5% Triton-X-100 in PBS to samples and incubate for 5 min at room temperature.  
*Note: Alternatively, use ice-cold 100% methanol for permeabilization.*
- Wash samples twice with PBS.

### Blocking

- Add 4% BSA in PBS to samples and incubate for 20 min at room temperature.  
*Note: If necessary, use additional blocking reagents (e.g. 10% normal serum in PBS or Image-iT™ FX Signal Enhancer) and extend the blocking time up to 60 min.*

### Incubation

- Dilute Spot-Label 1:800 in 4% BSA in PBS and incubate overnight at +4°C/+40°F.  
*Note: For multiplexing protocols, you can combine Spot-Label with another primary or secondary antibody.  
Optional: Incubate for 1-2 hours at room temperature instead of overnight at +4°C/+40°F.*
- Wash samples three times for 5-10 min in PBS.
- If required, counterstain with DNA fluorescent dyes, e.g. DAPI in PBS. Proceed with imaging directly or mount samples, if necessary.

### Mounting

- Rinse sample briefly in water to prevent salt crystal formation.
- Mount in ProLong™ Diamond Antifade Mountant or other mounting media with anti-fading agents.

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## Product overview and related products

Spot-Tag® toolbox	Product code
Spot-Trap® Agarose	eta-10; -20; -100
Spot-Trap® Agarose Kit	etak-20
Spot-Trap® Magnetic Agarose	etma-10; -20; -100
Spot-Trap® Magnetic Agarose Kit	etmak-20
Spot-Trap® Magnetic Particles M-270	etd-10; -20; -100
Spot-Trap® Magnetic Particles M-270 Kit	etdk-20
iST Spot-Trap® Kit (for IP/MS)	etak-iST-8
Binding Control Agarose	bab-20
Binding Control Magnetic Agarose	bmab-20
Spin columns	sct-10; sct-20; sct-50
Spot VHH, recombinant binding protein (bivalent)	etb-250
Spot-Cap®	eca-2
Spot-peptide	ep-1; -10
Spot-Cap® and peptide	eca-ep
Spot-Label® Alexa Fluor® 488	ebAF488-10; -50
Spot-Label® Alexa Fluor® 568	ebAF568-10; -50
Spot-Label® Alexa Fluor® 647	ebAF647-10; -50
Spot-Label® ATTO 488	eba488-10; -50
Spot-Label® ATTO 594	eba594-10; 50
pSpot1 ( <i>E. coli</i> , Spot N-term., Kan., high expression)	ev-1
pSpot2 ( <i>E. coli</i> , Spot C-term., Kan., high expression)	ev-2
pSpot3 ( <i>E. coli</i> , Spot C-term., Amp., low expression)	ev-3
pSpot4 ( <i>E. coli</i> , Spot N-term., Amp., low expression)	ev-4
pSpot5 ( <i>S. cerevisiae</i> , Spot N-term., Leu, CEN, low expression)	ev-5
pSpot6 ( <i>S. cerevisiae</i> , Spot C-term., Leu, CEN, low expression)	ev-6
pSpot7 ( <i>S. cerevisiae</i> , Spot N-term., Leu, 2 $\mu$ , high expression)	ev-7
pSpot8 ( <i>S. cerevisiae</i> , Spot C-term., Leu, 2 $\mu$ , high expression)	ev-8
pSpot Spot-Tag®-Actin (vector for expression of Spot-Tag® $\beta$ -actin fusion protein in mammalian cells)	ev-31
pSpot2 GFP-Spot-Tag® (vector for expression of GFP-Spot-Tag® fusion protein in <i>E. coli</i> )	ev-32
pSpot8 GFP-Spot-Tag® (vector for expression of GFP-Spot-Tag® fusion protein in <i>S. cerevisiae</i> )	ev-33

For product details, information, and ordering visit [www.chromotek.com](http://www.chromotek.com) and [www.ptglab.com](http://www.ptglab.com).

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## Disclaimer

Only for research applications, not for diagnostic or therapeutic use!

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