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Datasheet for ABIN5542492

## anti-TLR9 antibody (AA 868-1016)

2 Validations

7 Images

### Overview

Quantity:	0.1 mg
Target:	TLR9
Binding Specificity:	AA 868-1016
Reactivity:	Human
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This TLR9 antibody is un-conjugated
Application:	Western Blotting (WB), Immunohistochemistry (IHC), ELISA, Flow Cytometry (FACS), Immunocytochemistry (ICC)

### Product Details

Immunogen:	Purified recombinant fragment of human TLR9 (AA: 868-1016) expressed in E. coli.
Clone:	1B12H2
Isotype:	IgG2a
Purification:	purified

### Target Details

Target:	TLR9
Alternative Name:	TLR9 ( <a href="#">TLR9 Products</a> )
Background:	Description: The protein encoded by this gene is a member of the Toll-like receptor (TLR) family

## Target Details

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which plays a fundamental role in pathogen recognition and activation of innate immunity. TLRs are highly conserved from *Drosophila* to humans and share structural and functional similarities. They recognize pathogen-associated molecular patterns (PAMPs) that are expressed on infectious agents, and mediate the production of cytokines necessary for the development of effective immunity. The various TLRs exhibit different patterns of expression. This gene is preferentially expressed in immune cell rich tissues, such as spleen, lymph node, bone marrow and peripheral blood leukocytes. Studies in mice and human indicate that this receptor mediates cellular response to unmethylated CpG dinucleotides in bacterial DNA to mount an innate immune response.,

Aliases: CD289

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Molecular Weight: 115.8 kDa

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Gene ID: 54106

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HGNC: 54106

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Pathways: [TLR Signaling](#), [Activation of Innate immune Response](#), [Cellular Response to Molecule of Bacterial Origin](#), [Toll-Like Receptors Cascades](#)

## Application Details

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Application Notes: ELISA: 1:10000, WB: 1:500 - 1:2000, IHC: 1:200 - 1:1000, ICC: 1:200 - 1:1000, FCM: 1:200 - 1:400

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Restrictions: For Research Use only

## Handling

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Format: Liquid

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Buffer: Purified antibody in PBS with 0.05 % sodium azide

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Preservative: Sodium azide

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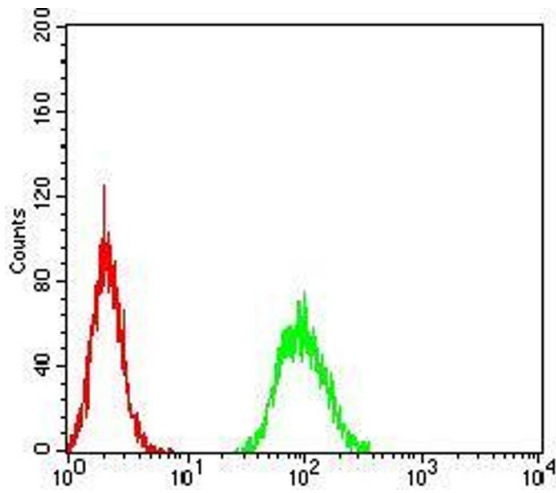
Precaution of Use: This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.

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Storage: 4 °C/-20 °C

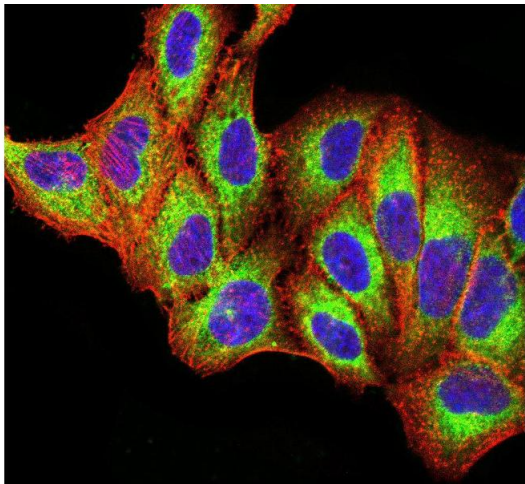
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Storage Comment: 4°C, -20°C for long term storage



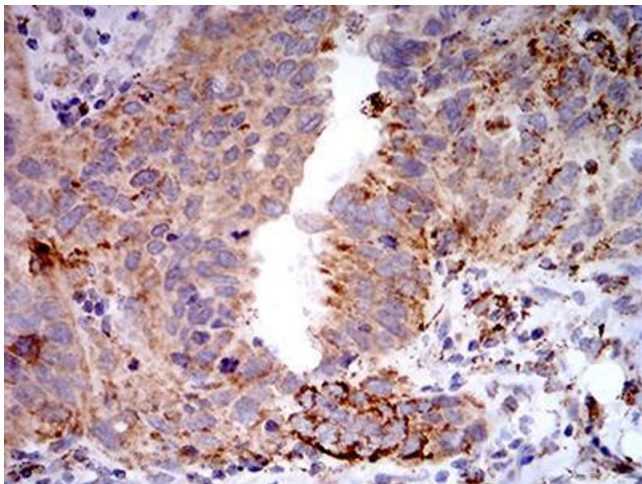
### Flow Cytometry

**Image 1.** Flow cytometric analysis of A549 cells using TLR9 mouse mAb (green) and negative control (red).



### Immunocytochemistry

**Image 2.** Immunofluorescence analysis of SK-OV-3 cells using TLR9 mouse mAb (green). Blue: DRAQ5 fluorescent DNA dye. Red: Actin filaments have been labeled with Alexa Fluor- 555 phalloidin. Secondary antibody from Fisher



### Immunohistochemistry

**Image 3.** Immunohistochemical analysis of paraffin-embedded ovarian cancer tissues using TLR9 mouse mAb with DAB staining.

Please check the [product details page](#) for more images. Overall 7 images are available for ABIN5542492.



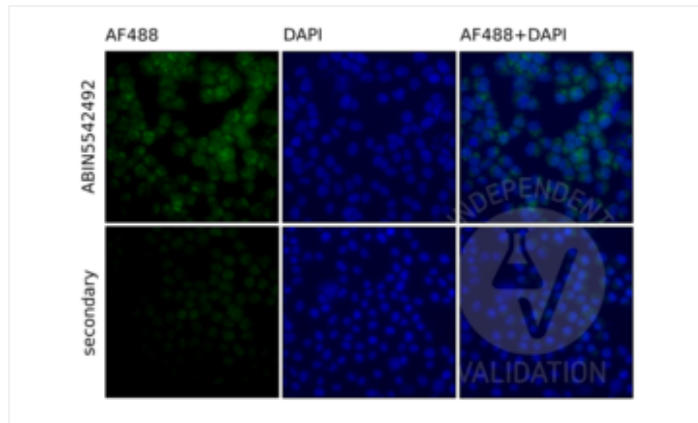
### Successfully validated (Immunofluorescence (IF))

by [Klinik für Anästhesie, Intensivmedizin und Schmerztherapie, Universitätsklinikum der Ruhr Universität Bochum](#)

Report Number: 103693

Date: Mar 26 2019

Target:	TLR9
Lot Number:	160127
Method validated:	Immunofluorescence (IF)
Positive Control:	U937 monocytic cells
Negative Control:	No primary antibody control
Notes:	Passed. ABIN5542492 localization on ER is visible. Negative control without primary antibody shows no signal.
Primary Antibody:	ABIN5542492
Secondary Antibody:	AF488-conjugated AffiniPure alpaca anti-Mouse IgG antibody (Jackson ImmunoResearch, 615-545-214)
Protocol:	<ul style="list-style-type: none"><li>• Grow U937 cells in RPMI (Gibco, 21875-034) supplemented with 10% FCS (Gibco, A3840001) and Pen/Strep(Gibco, 15140-122), at 37°C and 5% CO<sub>2</sub> in 500µL in 12-well plates.</li><li>• Use cytopspin to spin cells onto slide.</li><li>• Fix cells on coverslide in 4% PFA for 20min at RT.</li><li>• Wash cells 3x for 5min with PBS.</li><li>• Permeabilize cells in PBS containing 0.1% Triton for 5min at RT.</li><li>• Block non-specific binding with Duolink PLA Blocking Solution (Sigma) for 30 min at RT.</li><li>• Incubate cells with primary mouse anti-TLR9 antibody (antibodies-online, ABIN5542492, lot 160127) diluted 1:200 in staining solution ON at 4°C.</li><li>• Wash cells 3x for 5min with PBS.</li><li>• Incubate cells with secondary AF488-conjugated AffiniPure alpaca anti-Mouse IgG antibody (Jackson ImmunoResearch, 615-545-214) diluted 1:400 in staining solution for 1h at RT.</li><li>• Wash cells 2x for 5min with PBS.</li><li>• Mount coverslips on glass slides in ProLong Gold antifade reagent (Invitrogen, P36935,1890418) containing DAPI.</li><li>• Image acquisition with an Olympus widefield microscope.</li></ul>
Experimental Notes:	TLR9 staining using ABIN5542492 shows typical localization peri-nuclear localization.



**Validation image no. 1 for anti-Toll-Like Receptor 9 (TLR9)  
(AA 868-1016) antibody (ABIN5542492)**

U937 monocytic cells stained with with ABIN5542492 (top row). TLR9 staining (AF488, green) shows typical perinuclear localization (ER). Counterstain with DAPI (blue). Negative control without primary antibody (bottom row) shows no significant staining. Counterstain with DAPI (blue).



**Successfully validated (Proximity Ligation Assay (PLA))**

by [Klinik für Anästhesie, Intensivmedizin und Schmerztherapie, Universitätsklinikum der Ruhr Universität Bochum](#)

Report Number: 103821

Date: Apr 29 2019

Target:	TLR9
Lot Number:	160127
Method validated:	Proximity Ligation Assay (PLA)
Positive Control:	U937 monocytic cells stimulated with ODN1826
Negative Control:	Anti TLR9 antibody (ABIN5542492) alone Anti pY antibody (ABIN361758) alone
Notes:	Passed. PLA signal visible in stimulated cells. Negative control with only one primary antibody shows no signal.
Primary Antibody:	ABIN5542492
Secondary Antibody:	ABIN361758
Protocol:	<ul style="list-style-type: none"><li>• Grow U937 cells in RPMI (Gibco, 21875-034) supplemented with 10% FCS (Gibco, A3840001) and Pen/Strep(Gibco, 15140-122), at 37°C and 5% CO<sub>2</sub> in 500µL in 12-well plates.</li><li>• Incubate the cells with 1µM ODN1826 for 4h.</li><li>• Use cytospin to spin cells onto slide.</li><li>• Fix cells on coverslide in 4% PFA for 20min at RT.</li><li>• Wash cells 3x for 5min with PBS.</li><li>• Permeabilize cells in PBS containing 0.1% Triton for 5min at RT.</li><li>• Block non-specific binding with Duolink PLA Blocking Solution (Sigma-Aldrich) for 30min at RT.</li><li>• Incubate cells with primary mouse anti-TLR9 antibody (antibodies-online, ABIN5542492, lot 160127) diluted 1:200 and primary rabbit anti-pY antibody (antibodies-online, ABIN361758, lot 1508290) diluted 1:100 ON at 4°C.</li><li>• Wash cells 3x for 5min with PBS.</li><li>• Incubate cells with Duolink In Situ PLA Probe anti-Rabbit MINUS (Sigma-Aldrich, DUO92005, lot SLBZ4516) and Duolink In Situ PLA Probe anti-Mouse mouse PLUS (Sigma-Aldrich, DUO92001, lot SLBZ8369) proximity probes according to manufacturers recommendations.</li><li>• Wash cells 2x for 5min with PBS.</li><li>• Perform Ligation step according to the Duolink PLA kit (Sigma).</li><li>• Perform Amplification step according to the Duolink In Situ PIA Detection Kit Green (Sigma-Aldrich, DUO92014).</li></ul>

## Validation report #103821 for Proximity Ligation Assay (PLA)

- Mount coverslips on glass slides in ProLong Gold antifade reagent (Invitrogen, P36935, lot 1890418) containing DAPI.
- Image acquisition with an Olympus widefield microscope.

Experimental Notes: We used double the amount the Phi29 polymerase for the amplification step than recommended.

### Image for Validation report #103821



#### Validation image no. 1 for anti-Toll-Like Receptor 9 (TLR9) (AA 868-1016) antibody (ABIN5542492)

Negative controls of U937 cells without either ABIN5542492 or ABIN361758 show no signal. In U937 cells incubated simultaneously with ABIN5542492 and ABIN361758 the PLA signal (green) is clearly visible. Counterstain with DAPI (blue).