Product Datasheet

TLR7 Antibody (4G6) - BSA Free
NBP2-27332

Unit Size: 0.1 mg

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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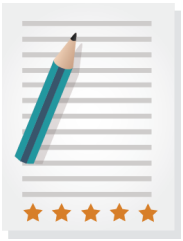
Updated 10/23/2024 v.20.1



NBP2-27332

TLR7 Antibody (4G6) - BSA Free

Product Information

Unit Size	0.1 mg
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	4G6
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS

Product Description

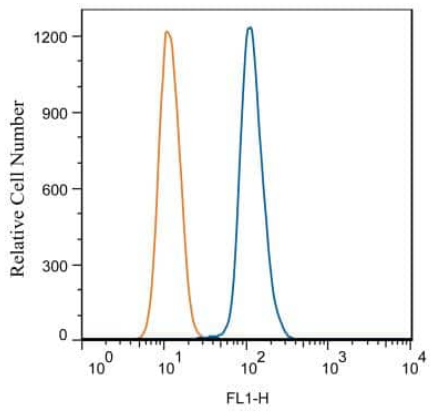
Host	Mouse
Gene ID	51284
Gene Symbol	TLR7
Species	Human, Mouse
Reactivity Notes	Mouse reactivity reported in scientific literature (PMID: 24126361)
Immunogen	A partial human TLR7 recombinant protein (amino acids 562-839) was used as the immunogen.

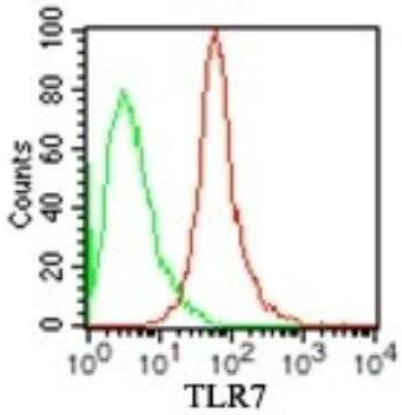
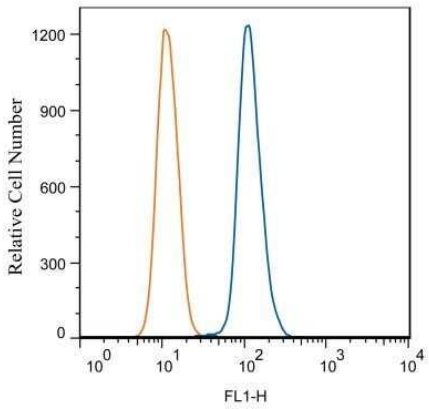
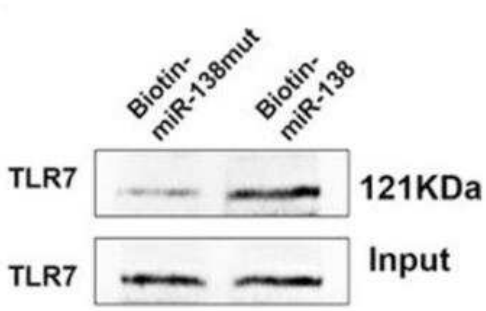
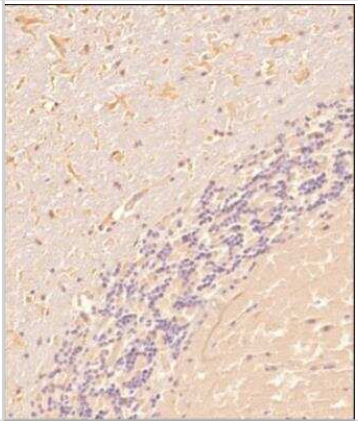
Product Application Details

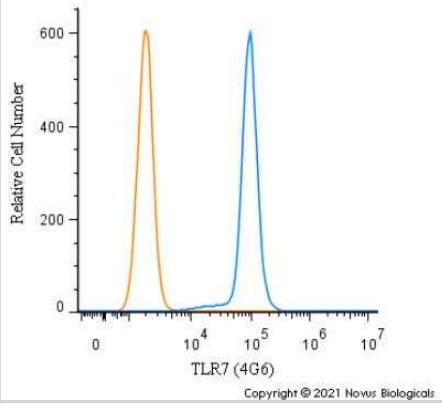
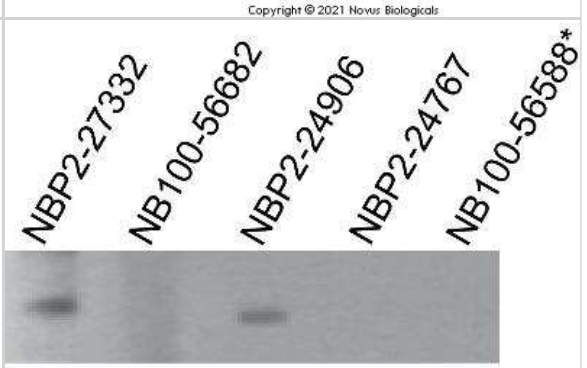
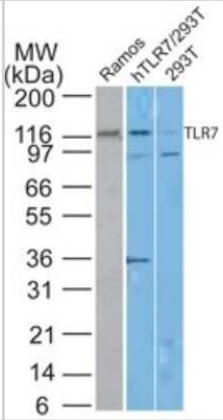
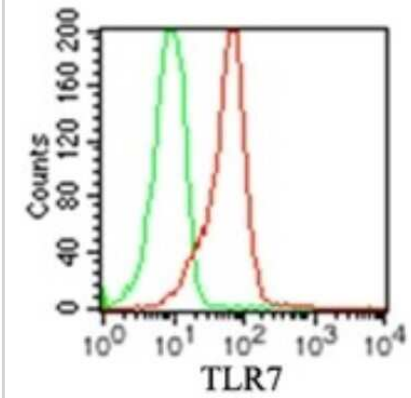
Applications	Western Blot, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, CyTOF-ready, Knockdown Validated, Knockout Validated
Recommended Dilutions	Western Blot 10ug/ml~, Flow Cytometry 1-2ug/10^6cells, Immunohistochemistry 1:50 - 1:200. Use reported in scientific literature (PMID 31092820), Immunocytochemistry/ Immunofluorescence 1:500-1:5000. Use reported in scientific literature (PMID 22610069), Flow (Intracellular) 5 ug/ml, CyTOF-ready, Knockout Validated, Knockdown Validated reported in scientific literature (PMID 31730654)
Application Notes	This antibody is CyTOF ready.

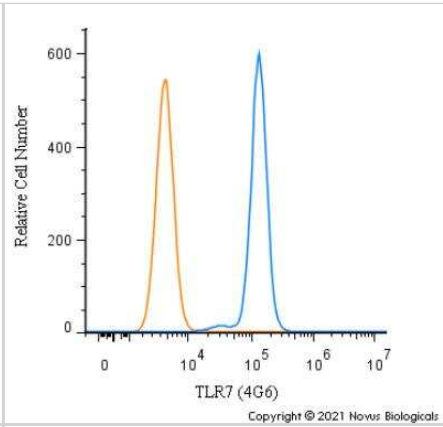
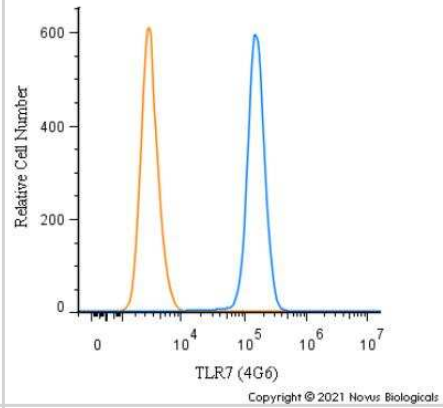
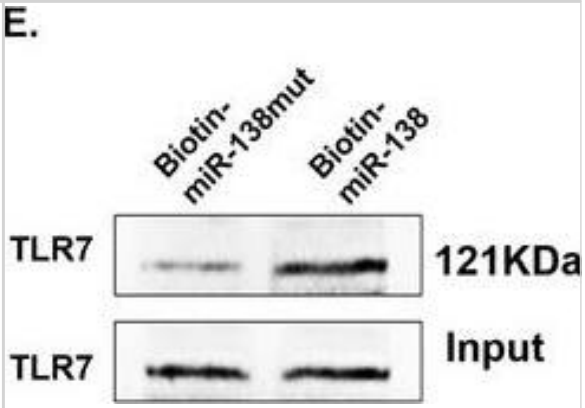
Images

Flow Cytometry: TLR7 Antibody (4G6) [NBP2-27332] - THP-1 cells were stained with TLR7 (4G) NBP2-27332 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature, followed by Dylight488-conjugated anti-mouse secondary antibody.



Flow (Intracellular): TLR7 Antibody (4G6) [NBP2-27332] - Analysis using the PE conjugate of NBP2-27332. Staining of TLR7 in 10^6 human BDCM cells (red) and 0.5 ug of mouse IgG1 isotype control (green). this antibody was used for this test.	
Flow Cytometry: TLR7 Antibody (4G6) [NBP2-27332] - Analysis using Azide Free version of NBP2-27332. THP-1 cells were stained with TLR7 (4G) NBP2-25274 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin.	
Western Blot: TLR7 Antibody (4G6) - BSA Free [NBP2-27332] - The protein of TLR7 were pull down by miR-138-biotin / miR-mut-138-biotin with Streptavidin agarose beads in BV2 cells. Image collected and cropped by CiteAb from the following publication (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7710131/) licensed under a CC-BY license.	
Immunohistochemistry: TLR7 Antibody (4G6) [NBP2-27332] - Analysis of a FFPE tissue section of human brain using 1:200 dilution of TLR7 [4G6] antibody. The staining was developed using HRP labeled anti-rabbit secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin.	

Flow Cytometry: TLR7 Antibody (4G6) [NBP2-27332] - An intracellular stain was performed on Ramos cells with TLR7 Antibody (4G6) NBP2-27332 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (35503, Thermo Fisher).	
Western Blot: TLR7 Antibody (4G6) [NBP2-27332] - TLR7 antibodies tested at 2 ug/ml on recombinant partial hTLR7 protein (amino acids 562 -839). NB100-56682 and NBP2-24767 immunogen sequences are not present in recombinant protein; *NB100-56588 is not recommended for use in western blot.	
Western Blot: TLR7 Antibody (4G6) [NBP2-27332] - Analysis using Azide Free version of NBP2-27332. Human TLR7 antibody in Ramos and transfected 293T lysate using TLR7 monoclonal antibody at 10 ug/ml.	
Flow Cytometry: TLR7 Antibody (4G6) [NBP2-27332] - Analysis of TLR7 in human monocytes using 2 ug of TLR7 monoclonal antibody (red) and 2 ug of mouse IgG1 isotype control antibody (green). TLR intracellular flow kit was used for this test, and an anti-mouse IgG PE conjugated secondary antibody .	

Flow Cytometry: TLR7 Antibody (4G6) [NBP2-27332] - An intracellular stain was performed on Daudi cells with TLR7 Antibody (4G6) NBP2-27332 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (35503, Thermo Fisher).	
Flow Cytometry: TLR7 Antibody (4G6) [NBP2-27332] - An intracellular stain was performed on Raji cells with TLR7 Antibody (4G6) NBP2-27332 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (35503, Thermo Fisher).	
Western Blot: TLR7 Antibody (4G6) - BSA Free [NBP2-27332] - ADEV miR□138 interacts with murine TLR7 in the endosomes. (A) Representative fluorescence images of mouse primary microglial cells incubated with Exo□Fect+ADEVs+Cy5□miR138 or Exo□Fect +Cy5□miR138 (without ADEVs) or Exo□Fect+ADEVs+miR138 (unstained) for 30 min followed by immunostaining of (A) an early endosome marker (EEA1, Green) & (B) TLR7 (Green). Exo□fect +ADEVs+Cy5□miR138, Cy5□miR138 were loaded into ADEVs using by Exo□Fect transfection kit; Exo□fect+Cy5□miR138, Cy5□miR138 were loaded using by Exo□Fect transfection kit (without ADEVs); Exo□fect +ADEVs+miR138, unstained miR138 were loaded into ADEVs using Exo□Fect transfection kit; Bars, 50 μm (n = 3). (C) TLR7 was immunoprecipitated from BV2 cells by IgG / TLR7 / LAMP1 antibody, followed by assessment of miR138 / U6 expression by real□time PCR. One□way ANOVA followed by Bonferroni's post hoc test was used to determine the statistical significance among multiple groups (n = 3). (D) TLR7 was immunoprecipitated from HEK□Null / HEK□TLR7 cells by IgG/TLR7 antibody, followed by assessment of miR138 expression by real□time PCR. One□way ANOVA followed by Bonferroni's post hoc test was used to determine the statistical significance among multiple groups (n = 3). (E) The protein of TLR7 were pull down by miR□138□biotin / miR□mut□138□biotin with Streptavidin agarose beads in BV2 cells. All data are presented as mean ± SD or SEM of three individual experiments. *,P < 0.05; **,P < 0.01; ***,P < 0.001 versus control group. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/33304479), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Publications

Ivana Matic Girard, Paul Ward, Angela Durey, Stephan Lund, Hanny Calache, Sarah R Baker, Linda Slack-Smith Primary caregivers' perceptions of factors influencing preschool children's oral health: social practices perspective-a protocol for qualitative metasynthesis. BMJ open 2023-04-13 [PMID: 37041059]

Lin T, Hu L, Hu F et al. NET-Triggered NLRP3 Activation and IL18 Release Drive Oxaliplatin-Induced Peripheral Neuropathy Cancer Immunology Research 2022-12-02 [PMID: 36255412]

Wallach T, Mossmann ZJ, Szczepek M et al. MicroRNA-100-5p and microRNA-298-5p released from apoptotic cortical neurons are endogenous Toll-like receptor 7/8 ligands that contribute to neurodegeneration Molecular Neurodegeneration 2021-12-01 [PMID: 34838071]

Wang Z, Sun Y, Lou F et al. Targeting the transcription factor HES1 by L-menthol restores protein phosphatase 6 in keratinocytes in models of psoriasis Nature communications 2022-12-19 [PMID: 36535970] (WB, Mouse)

Details:
Dilution used in WB 1:100

Brown GJ, CaNete PF, Wang H Et al. TLR7 gain-of-function genetic variation causes human lupus Nature 2022-04-28 [PMID: 35477763] (FLOW, Human)

Details:
Citation using the PE version of this antibody.

Lou Z, Su R, Wang W et al. EV71 infection induces neurodegeneration via activating TLR7 signaling and IL-6 production PLoS Pathog 2019-11-15 [PMID: 31730654] (KD, KO, IHC-P, Mouse)

Lam LKM, Dobkin J, Eckart KA et al. Bat Red Blood Cells express Nucleic Acid Sensing Receptors and bind RNA and DNA Immunohorizons 2022-05-20 [PMID: 35595326]

Liao, K, Niu, F Et al. Morphine-mediated release of miR-138 in astrocyte-derived extracellular vesicles promotes microglial activation. J Extracell Vesicles 2020-10-01 [PMID: 33304479] (WB, Mouse)

Klammer MG, Dzaye O, Wallach T et al. UNC93B1 Is Widely Expressed in the Murine CNS and Is Required for Neuroinflammation and Neuronal Injury Induced by MicroRNA let-7b Frontiers in immunology 2021-09-13 [PMID: 34589086] (IF/IHC, Mouse)

Hagen SH, Henseling F, Hennesen J et al Heterogeneous Escape from X Chromosome Inactivation Results in Sex Differences in Type I IFN Responses at the Single Human pDC Level Cell Rep 2020-12-09 [PMID: 33296655]

Details:
Citation using the Allophycocyanin version of this antibody.

Kitaura A, Nishinaka T, Hamasaki S, et al. Advanced glycation end-products reduce lipopolysaccharide uptake by macrophages PloS one 2021-01-25 [PMID: 33493233]

Lou F, Sun Y, Xu Z et al. Excessive Polyamine Generation in Keratinocytes Promotes Self-RNA Sensing by Dendritic Cells in Psoriasis Immunity 2020-06-13 [PMID: 32553276] (ICC/IF, Mouse)

More publications at http://www.novusbio.com/NBP2-27332

Procedures

Western Blot Protocol for TLR7 Antibody (NBP2-27332)
1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructio

Flow (Intracellular) Protocol for TLR7 Antibody (NBP2-27332)

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2 x 105 and 1 x 106 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 uL for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.
a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.
4. Re-suspend cells to a concentration of 1 x 106 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 uL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeablization steps might reduce the availability of surface antigens.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

1. Fix the cells by adding 100 uL fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 uL of a permeabization buffer to every 1 x 106 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.
a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.
b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).
3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100 uL of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 ug per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.

Immunohistochemistry-Paraffin Protocol for TLR7 Antibody (NBP2-27332)
Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer all the time).

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.

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Products Related to NBP2-27332

NBP2-26228-1mg	Imiquimod, TLR7 ligand
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-43319-0.5mg	Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1)

Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

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