

# pZERO-mTLR9-HA

A plasmid expressing a HA-tagged TIR-deleted mouse TLR9 gene

Catalog # pzzero-mtlr9ha

For research use only

Version # 10A14-MM

## PRODUCT INFORMATION

### Content:

- 1 disk of lyophilized GT116 *E. coli* bacteria transformed pZERO-mTLR9-HA.
- GT116 genotype is: *F-, mcrA, Δ(mrr-hsdRMS-mcrBC), Ø80lacZΔM15, ΔlacX74, recA1, endA1 Δdcm ΔsbcC-sbcD*.
- 4 pouches of *E. coli* Fast-Media® Puro.

### Storage and stability:

- Products are shipped at room temperature.
- Transformed bacteria should be stored at -20°C and are stable up to 1 year.
- Store *E. coli* Fast-Media® Puro at room temperature. Fast-Media® is stable 18 months when stored properly.

### Quality control:

- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Bacteria have been lyophilized, and their viability upon resuspension has been verified.

## GENERAL PRODUCT USE

Toll-like receptors (TLRs) activate intracellular signaling pathways that share much in common with IL1-R signaling, owing to their conserved TIR (Toll/IL-1R) domains present in the cytoplasmic tails. The TIR family also includes the adapters MyD88, TIRAP (MAL) and a new member TRIF (TICAM). Upon ligand binding, TLRs recruit MyD88 via their TIR domains. The TLR/MyD88 complex then activates IRAK eliciting a signaling cascade leading to the activation of NF-κB. Some TLRs also signal through a MyD88-independent pathway. Recently, TLR3 and TLR4 have been reported to interact with TRIF inducing the activation of the IRF-3 signaling pathway. Despite immense progress in the understanding of the TLR pathways, a lot remains unclear. To help you uncover the complex mechanisms governing TLR signaling, InvivoGen has engineered TIR domain-deleted TLR genes (TLR-ΔTIR). These mutant genes will serve as useful tools to study TLR signaling.

All ten human and nine murine TLR-ΔTIR genes are available in **pZERO-TLR** plasmids. Their expression is controlled by the strong EF1/HTLV composite promoter. **pZERO-TLR** plasmids are selectable with the potent antibiotic puromycin in both *E. coli* and mammalian cells.

## PLASMID FEATURES

### • Murine TLR9-ΔTIR-HA (2643 bp)

TLR9, which is localized intracellularly, is involved in the recognition of specific unmethylated CpG-ODN sequences, that distinguishes bacterial DNA from mammalian DNA. TLR9 engages an intracellular pathway that involves MyD88 leading to NF-κB translocation<sup>1</sup>. TLR9 has been shown to recognize different CpG motifs; the optimal sequences being GTCGTT and GACGTT for hTLR9 and mTLR9 respectively<sup>2</sup>. The ectodomains of hTLR9 and mTLR9 present distinct distribution patterns of leucine-rich repeats. The sequence differences observed in the ectodomains of h- and m-TLR9 may account for the differences in ligand specificity in human versus murine TLR9.

TLR9-ΔTIR-HA is a TIR-less form of the TLR9 gene generated by deleting the TIR domain (489 bp) and adding the influenza hemagglutinin epitope tag (YPYDVPDYA) at the 3' end. The HA tag allows for simple and convenient detection of the expressed TLR-ΔTIR gene by Western blot using an HA primary antibody. InvivoGen offers an Anti-HA tag (catalog code: ab-hatag) that can be used to detect the expressed fusion protein.

- **EF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter<sup>4</sup> and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat<sup>4</sup>. EF-1α is a 'housekeeping' gene ubiquitously expressed in eukaryotic cells. The EF-1α promoter exhibits a strong activity, higher than viral promoters and, on the contrary to the CMV promoter, yields persistent expression of the transgene *in vivo*. The R-U5' has been coupled to the EF-1α core promoter to enhance stability of DNA and RNA.
- **SV40 pAn:** The SV 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.
- **Ori** is a minimal *E. coli* origin of replication with the same activity as the longer Ori.
- **CMV prom:** The human cytomegalovirus immediate-early gene 1 promoter/enhancer was originally isolated from the Towne strain and was found to be stronger than any other viral promoters.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Pac:** The *Pac* gene encodes a puromycin N-acetyl-transferase that confers resistance to the antibiotic puromycin. Mammalian cells are generally sensitive to concentrations ranging from 1 to 10 μg/ml depending on the cell line.
- **βGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription<sup>5</sup>.

## References

1. Chuang TH. & RJ. Ulevitch (2000). *Eur Cytokine Netw*, 11(3):372-8.
2. Hemmi H. *et al* (2002). *Nat Immunol*, 3(2):196-200.
3. Lee J *et al*. (2003). *Proc Natl Acad Sci U S A*, 100(11):6646-6651
4. Kim *et al*. (1990). *Gene* 2: 217-223.
5. Yu J & Russell JE. (2001). *Mol Cell Biol*, 21(17):5879-88.

## METHODS

### Growth of pZERO-transformed bacteria:

**Use sterile conditions** to do the following:

- 1- Resuspend the lyophilized *E. coli* by adding 1 ml of LB medium in the tube containing the disk. Let sit for 5 minutes. Mix gently by inverting the tube several times.
- 2- Streak bacteria taken from this suspension on a puromycin LB agar plate prepared with the *E. coli* Fast-Media® Puro agar provided (see below).
- 3- Place the plate in an incubator at 37°C overnight.
- 4- Isolate a single colony and grow the bacteria in TB supplemented with puromycin using the Fast-Media® Puro liquid provided (see below).
- 5- Extract the pZERO plasmid DNA using the method of your choice.

**Note:** For long-term storage of the pZERO-transformed bacteria, prepare a 20% glycerol stock of the bacteria grown in the overnight liquid culture and freeze at -80°C.

## TECHNICAL SUPPORT

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#### **Selection of bacteria with *E. coli* Fast-Media Puro:**

*E. coli* Fast-Media® Puro is a **new, fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave.

#### **Method:**

- 1- Pour the contents of a pouch into a clean borosilicate glass bottle or flask.
- 2- Add 200 ml of distilled water to the flask.
- 3- Heat in a microwave on MEDIUM power setting (about 400Watts), until bubbles start appearing (approximately 3 minutes). **Do not heat a closed container. Do not autoclave Fast-Media®.**
- 4- Swirl gently to mix the preparation. **Be careful, the bottle and media are hot, use heatproof pads or gloves and care when handling.**
- 5- Reheat the media for 30 seconds and gently swirl again. Repeat as necessary to completely dissolve the powder into solution. But be careful to avoid overboiling and volume loss.
- 6- Let agar medium cool to 45°C before pouring plates. Let liquid media cool to 37°C before seeding bacteria.

*Note: Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.*

#### **Preparation of cell extract for detection of HA-tagged® protein:**

The buffer used to prepare the cell lysates is a modified RIPA buffer that is suitable for recovery of membrane receptors such as the TLRs.

- 1- Lift cells from 6-well plate and wash twice with PBS.
- 2- Add 100 µl of Cell Lysis Buffer and incubate 30 min. on ice.
- 3- Centrifuge 10 min @ 10,000 rpm (at 4°C if possible).
- 4- Transfer supernatant to a new tube and store @-20°C.

#### **Cell Lysis Buffer**

- 5 ml of 100 mM Tris HCl, pH 7.4.
- 5 ml of 1M NaCl
- 100 µl of 0.5M EDTA
- 500 µl of 100mM NaF
- 500 µl of 10% SDS
- 2.5 ml of 10% Na-Deoxycholate
- 500 µl of 100% Triton-X 100
- 5 ml of 10% Glycerol
- H<sub>2</sub>O to a final volume of 50 ml.

Store this stock buffer @ 4°C.

Determine amount of lysis buffer needed to make cell extracts and just before use add the following components at the final concentrations below:

- 1 mM PMSF
- 1:100 Protease Inhibitor cocktail
- 2 mM of Na<sub>3</sub>VO<sub>4</sub>

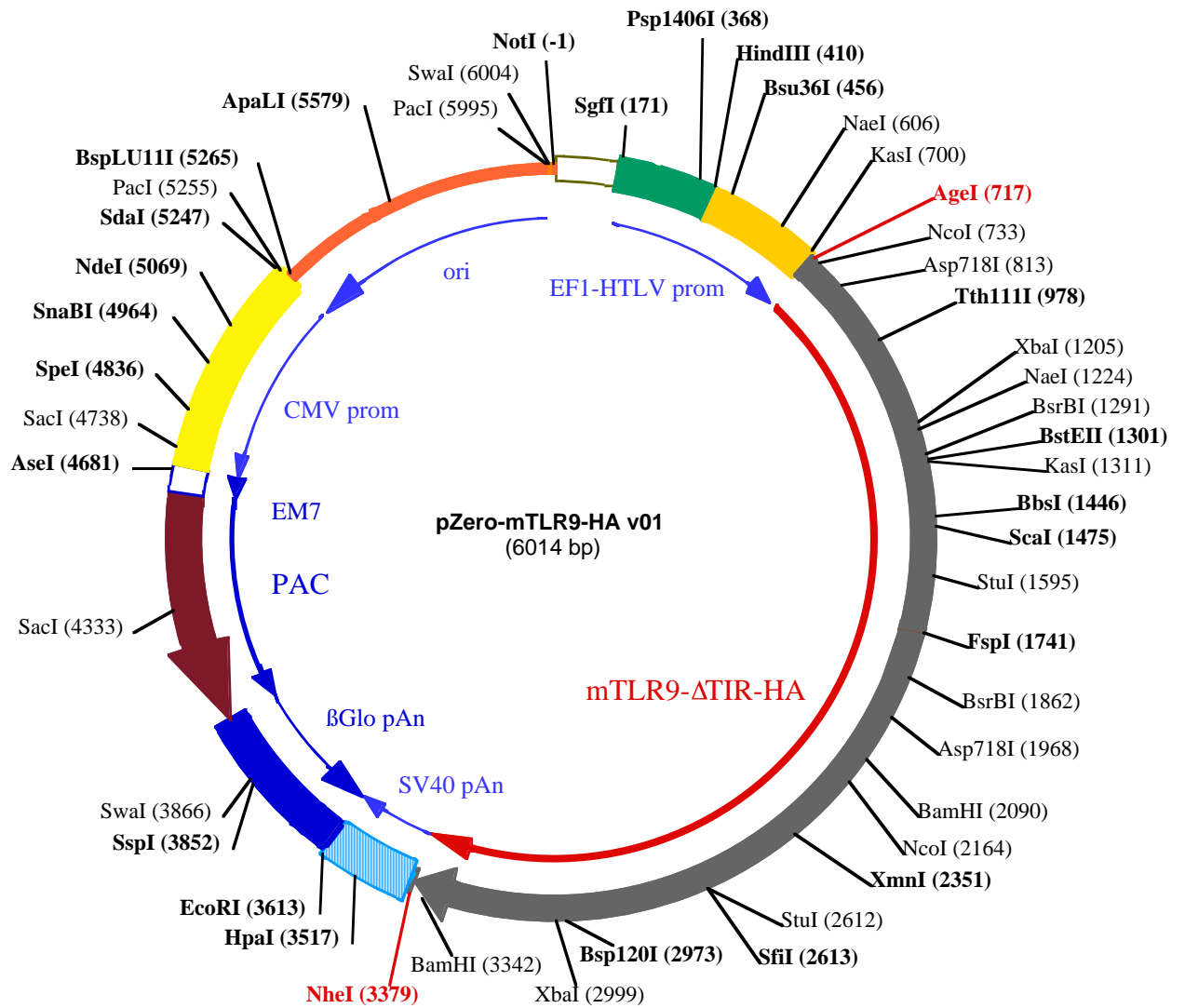
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2701 CTGAAGCTGCTGAGCCTCCGAGACAACTACCTATCTTTCTTTAACTGGACCAGTCTGTCTTCTGCCCCAACCCTGGAAGTCCTAGACCTGGCAGGCAACC  
656▶ L K L L S L R D N Y L S F F N W T S L S F L P N L E V L D L A G N  
2801 AGCTAAAGGCCCTGACCAATGGCACCCCTCCTCCAGAACTGGATGTCAGCAGCAACAGTATCGTCTCTGTGGTCCAGCCTTCTT  
689▶ Q L K A L T N G T L P N G T L L Q K L D V S S N S I V S V V P A F F  
Bsp120I (2973) XbaI  
2901 CGCTCTGGGGTCCGAGCTGAAAGAGGTCAACCTCAGCCACAACATTCTCAAGACGGTGGATCGCTCCTGGTTTGGGCCCATTTGTGATGAACCTGACAGTT  
722▶ A L A V E L K E V N L S H N I L K T V D R S W F G P I V M N L T V  
3001 CTAGACGTGAGAAGCAACCTCTGCACTGTGCTGTGGGGCAGCCTTCGTAGACTTACTGTGGAGGTGAGACCAAGGTGCTGGCTGGCTAATGGTG  
756▶ L D V R S N P L H C A C G A A F V D L L L E V Q T K V P G L A N G  
3101 TGAAGTGTGGCAGCCCGGCCAGCTGCAGGGCCGTAGCATCTTCGCAACAGGACCTGCGGCTGTGCCTGGATGAGGTCCTCTCTGGGACTGCTTTGGCCT  
789▶ V K C G S P G Q L Q G R S I F A Q D L R L C L D E V L S W D C F G L  
3201 TTCACCTCTGGCTGTGGCCGTGGGCATGGTGGTCCCTATACTGCACCATCTCTGCGGCTGGGACGCTCTGGTACTGTTTTCATCTGTGCCTGGCATGGCTA  
822▶ S L L A V A V G M V V P I L H H L C G W D V W Y C F H L C L A W L  
BamHI (3342) NheI (3379)  
3301 CCTTGTCTGGCCCGCAGCCGACGCGAGCCCAAGCTCTCCCGGATCCTACCCCTATGATGTGCCAGACTACGCCTAAAGCTAGCTGGCCAGACATGATA  
856▶ P L L A R S R R S A Q A L P G S Y P Y D V P D Y A •  
3401 AGATACATTTGATGAGTTTGGACAAACCACAACCTAGAAATGCAGTGAACCAATTTGATGATGCTATTGCTTTTATTGTAACCATTA  
HpaI (3517)  
3501 TAAGCTGCAATAAACAGTTAACAAACAATTCGATTCATTTTATGTTTCAGGTTTCAGGGGGAGGTGTGGGAGGTTTTTTTAAAGCAAGTAAACCTCTA  
EcoRI (3613)  
3601 CAAATGTGGTATGGAATTTCTAAAATACAGCATAGCAAACTTTAACTCCAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGG  
3701 CATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAAGATATAGTGTATTTTCCCAAGTTTGAAGTAGCTCT  
SspI (3852) SmaI (3866)  
3801 TCATTTCTTTTATGTTTTAAATGCACTGACCTCCACATTCCTTTTTTAGTAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAATAAATGTT  
3901 TTTTATTAGCAGAAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTATGATGTTGAGCTTAGGGAAACAAAGGAACCTTTAATAGAAATTTGGACA  
4001 GCAAGAAAGCGAGCTTCTAGCTCAGGTTTAAAGCTCCAGGCTTCTTGTGCATGCACCAAGTTCTTGGGCTTCTGGAACCTCAACATCAGCTGTCCAGTG  
202▶ • T • A G P K R T M C W T R P G E P V E V D A T V T  
4101 AATCCAGCTTTTCATAAAAAGCGAGTTTCTGGGAGCAGAAGTTTCCAGAAAGGAGGAACTCCAGCCCTTTCCAGCAGCTTCAACTCCAGGCAGAAACA  
175▶ F G L R E Y F P L N R P A S T E L F A P V G A R E A A E V G P L V V  
4201 CAGCAGATCCAGACCCCTTCCCTGGTGGTCCAGGCTCACTCCAAAGTGGCCAGAAACCAAGCTGGCTCTTTTGGCCTGTGTGGTCCAGCAGACCTTC  
142▶ A S G L G K G Q H D P S V G V T A L F W A P E K P R H P A L L G E  
SacI (4333)  
4301 CATTTGTTGTTGTGCTGCCAGCCTGCTTCCAGAGAGCTCAGCCATTCTTGGTCCAATTTCCAGAAAAACAGCACCAGCTTCAACAGACTCAGGTGTGTGC  
109▶ M Q Q Q A A L R S G S L E A M R P G I E A F V A G A E V S E P T T  
4401 CAAATGCAACAGCAGCTCCATCATCTGCAACCCAAACTTTTCCAATGTCAGTCCCACTCTGGTGGGAAAGAGTTCTTGCAGTTCTGTCAACCTCTCAA  
75▶ W V A V A A G D D A V W V K G I D L G V R T L F L E Q L E T V R E I  
4501 TGTGCCTGTCCAGGTCAACTGTGTGCCTTGTTCAGGGTAGTCTGCAAAAGCAGCAGCCAGTGTCTCACAGCTCTTGGACATCATCTCTGTGTTGCCAG  
42▶ H R D P D V T H R T A P Y D A F A A A L T R V A R P V D R T A L  
AseI (4681)  
4601 CCTCACTGTGGGTTTGTACTCAGTCATGGTGGCCCTCCTATAGTGTGAGTCTATTATAGTATGCGGATATACTATGCCGATGATTAATTTGTCAAACAGCG  
9▶ R V T P K Y E T M  
SacI (4738)  
4701 TGGATGGCGTCTCCAGCTTATCTGACGGTTCACTAAACGAGCTCTGCTTATATAGACCTCCACCGTACAGCCTACCGCCATTTGCGTCAATGGGGCG  
SpeI (4836)  
4801 GAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTACTAGTCAAAACAACTCCCATTTGACGTCAATGGGGTGGAGACTTGGAAATCCCGTGAAGTCAA  
SnaBI (4964)  
4901 ACCGCTATCCACGCCCAATGATGTAAGTCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTAAGTCCCAAGTGGAAAGTCCCATAA  
NdeI (5069)  
5001 GGTCATGTAAGTGGCATAATGCCAGGCGGGCCATTTACCGTCAATGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGAATGTAAGTCCCAAGTGG  
5101 GCAGTTTACCGTAAATACTCCACCAATGACGTCAATGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGCGGGGGT  
PacI (5255)  
5201 CGTTGGGCGGTCCAGCCAGCGGGCCATTTACCGTAAAGTTATGTAACGCGCTGAGGTTAATTAGAAACATGTGAGCAAAAGGCCAGAAAGCCAGGAAC  
SdaI (5247) BspLU11I (5265)  
5301 CGTAAAAAGCGCGTGTGTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAG  
5401 GACTATAAAGATACCAGCGTTTTCCCTCGAAGCTCCCTCGTGGCTCTCTGTTCGACCCCTGCGGCTTACCGGATACCTGTCCGCTTTCTCCCTTC  
ApaLI (5579)  
5501 GGGAAAGCGTGGCGCTTTCTCATAGCTCAGCTGTAGTATCTCAGTTCGGTGTAGGTCGTTGCTCCAAGCTGGGCTGTGTGCAGCAACCCCGGTTCCAG  
5601 CCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGTAGTCCAAACCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAAACAGGATTAGCA

5701 GAGCGAGGTATGTAGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTGGTATCTGCGCTCTGCTGAAGCC

---

5801 AGTTACCTTCGGAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGA

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PacI (599)

5901 AAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACACGTTAAGGGATTTGGTCAITGGCTAGTTAAT

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SwaI (6004)

6001 TAACATTTAAATCA