Technical Data Sheet

Purified Mouse Anti-Human Toll-Like Receptor 4

Product Information

Material Number: 551964
Alternate Name: TLR4; CD284; Toll-like Receptor 4; ARMD10
Size: 0.1 mg
Concentration: 0.5 mg/ml
Clone: HTA125
Immunogen: Human TLR4-transfected Ba/F3 cell line
Isotype: Mouse (BALB/c) IgG2a, κ
Reactivity: QC Testing: Human
Storage Buffer: Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The HTA125 monoclonal antibody specifically binds to the human Toll-like receptor 4 (TLR4), also known as CD284. Toll-like receptors (TLR) are type I transmembrane proteins characterized by an extracellular domain containing leucine-rich repeats and a cytoplasmic domain similar to IL-1R family. In humans, ten TLR have been identified so far. The TLR family acts as a pattern-recognition receptor that is an essential component of pathogen recognition and innate immunity. TLR4 has been identified as the receptor for Gram-negative bacterial lipopolysaccharide (LPS). TLR4 mRNA expression has been found in spleen, placenta, ovary, intestine and lung. Using HTA125, TLR4 protein expression has been detected in monocytes, B cells and T cells in human peripheral blood. The human TLR4 gene has been mapped to chromosome 9q32. The immunogen used to generate the HTA125 hybridoma was human TLR4-transfected Ba/F3 cells. The HTA125 antibody reportedly blocks LPS-triggered, TLR4-mediated stimulation of cells.

Preparation and Storage

Store undiluted at 4°C. The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Application Notes

Application

<table>
<thead>
<tr>
<th>Flow cytometry</th>
<th>Routinely Tested</th>
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<tbody>
<tr>
<td>Blocking</td>
<td>Reported</td>
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Recommended Assay Procedure:

A multistep staining procedure is recommended to amplify immunofluorescent signals for the flow cytometric analysis of human TLR4 expression:

Step 1: Incubate 10^6 cells with 0.25 - 1.0 µg/test of purified HTA125 antibody at 4°C for at least 15 - 20 minutes. Wash cells two times with staining medium containing sodium azide (e.g., BD Pharmingen™ Stain Buffer, Cat. No. 554656).

Step 2: Incubate the cells with biotinylated anti-mouse IgG (Cat. No. 553999) or biotinylated anti-mouse IgG2a, (Cat. No. 553388) at 4°C for 20 minutes. Wash cells two times.

Step 3: Incubate the cells with ≤0.06 µg of streptavidin-phycoerythrin (Cat. No. 554061) at 4°C for 20 minutes. Wash two times. Resuspend cells.
in stain buffer and analyze stained cells by flow cytometry using appropriate specificity and compensation controls.

Suggested Companion Products

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
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</thead>
<tbody>
<tr>
<td>555571</td>
<td>Purified Mouse IgG2a, κ Isotype Control</td>
<td>0.1 mg</td>
<td>G155-178</td>
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<tr>
<td>553999</td>
<td>Biotin Goat Anti-Mouse Ig (Multiple Adsorption)</td>
<td>0.5 mg</td>
<td>Polyclonal</td>
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<tr>
<td>554061</td>
<td>PE Streptavidin</td>
<td>0.5 mg</td>
<td>(none)</td>
</tr>
<tr>
<td>555397</td>
<td>FITC Mouse Anti-Human CD14</td>
<td>100 Tests</td>
<td>M5E2</td>
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<tr>
<td>553388</td>
<td>Biotin Rat Anti-Mouse IgG2a</td>
<td>0.5 mg</td>
<td>R19-15</td>
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<tr>
<td>554656</td>
<td>Stain Buffer (FBS)</td>
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<tr>
<td>555899</td>
<td>Lysing Buffer</td>
<td>100 mL</td>
<td>(none)</td>
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</tbody>
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Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
3. An isotype control should be used at the same concentration as the antibody of interest.

References


