### Overview

<table>
<thead>
<tr>
<th>Product name</th>
<th>Human Rheumatoid factor and IgM Clean-up Solution (ab215121)</th>
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</thead>
<tbody>
<tr>
<td><strong>Product overview</strong></td>
<td>Human Rheumatoid factor and IgM Clean-up Solution (ab215121) is designed to help alleviate Rheumatoid factor and IgM interference by removal of the proteins from the serum/plasma sample prior to addition into the FirePlex immunoassay. The use of Human Rheumatoid factor and IgM Clean-up Solution (ab215121) dramatically helps particle recovery in high RF+ or Rheumatoid arthritis serum/plasma samples, allowing for a functional assay. The Clean-up solution is suitable for all FirePlex immunassay panels.</td>
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The presence of elevated concentrations of autoantibodies in biological samples are known to cause interference in a number of immunological assays for the measurement of biomarkers and cytokines [1, 2]. One important group of interfering heterophilic antibodies are rheumatoid factor (RF), defined as autoimmune antibodies directed against the Fc region of an IgG, present in 65-80% of patients with rheumatoid arthritis (RA), in pneumonia, and in a number of arthritic and connective tissue diseases [1]. RF can exist as either IgA, IgD, IgE, IgG, or IgM, however the predominant forms are IgM and IgG [1]. Reduction of the levels of these interfering factors is important for accurate measurement of biomarkers and cytokines by immunoassay. This kit has been designed for the removal of RF and IgM from human biological samples such as serum and plasma.

### References


### Notes

**Protocol for using human Rheumatoid factor and IgM Clean-up Solution:**
**Plasma:** Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Plasma samples can be treated using this kit immediately after collection or may be stored at -20°C or below for future treatment for up to 3 months. Avoid repeated freeze-thaw cycles.

**Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Serum samples can be treated using this kit immediately after collection or may be stored at -20°C or below for future treatment for up to 3 months. Avoid repeated freeze-thaw cycles.

1. Treatment can be used for serum or plasma samples (fresh or thawed)
2. Clean-up Solution is provided at 10X concentration, add to samples in a 1:10 ratio for a final concentration of 1X

   Example; add 20 µL 10X Clean-up Solution to 180 µL sample
   Example; add 50 µL 10X Clean-up Solution to 450 µL sample
   Example; add 100 µL 10X Clean-up Solution to 900 µL sample

   Note: a white precipitate may form
3. Mix by pipetting and incubate samples at 4°C for 1 hour
4. Centrifuge at 700 x g for 45 minutes at 4°C
5. Carefully transfer supernatant to a fresh tube, ensuring that the pellet and any precipitates are not disturbed (pellets can be discarded)
6. For further clarification, centrifuge the supernatants at 2,000 x g for 15 minutes at 4°C
7. Carefully transfer supernatant to a fresh tube, ensuring that the pellet and any precipitates are not disturbed (pellets can be discarded)
8. Assay samples immediately or aliquot and store at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

**Properties**

<table>
<thead>
<tr>
<th>Storage instructions</th>
<th>Store at +4°C. Please refer to protocols.</th>
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<tr>
<th>Components</th>
<th>1 units</th>
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<tbody>
<tr>
<td>10X Rheumatoid factor and IgM Clean-up Solution</td>
<td>1 x 1ml</td>
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</table>

**Cellular localization**

Rheumatoid Factor: Secreted IgM: Isoform 1: Secreted. During differentiation, B lymphocytes switch from expression of membrane bound IgM to secretion of IgM. Isoform 2: Cell membrane; Single pass type I membrane protein.

**Images**
The Rheumatoid factor and IgM Clean-up Kit was used for the clarification of sera from patients with rheumatoid arthritis (RA) containing low, medium and high concentrations of rheumatoid factor (RF), alongside a sample of pooled normal human serum and plasma as controls. Untreated and treated samples were then used for the analysis of six cytokines in duplicate using Firefly® Multiplex Immunoassay kits; TNF alpha (ab208205), IL-9 (ab209506), IL-13 (ab208210), IL-17A (ab208215), MCP1 (ab208211), IL-1Ra (ab208224). Recovery of Firefly® particles for each analyte and sample type was calculated as a percentage of the recovery in untreated control serum or plasma. For untreated sera from RA patients, particle recovery and therefore assay performance inversely correlates with RF concentration. Upon treatment with the Rheumatoid factor and IgM Clean-up Kit, particle recovery was restored.

The Rheumatoid factor and IgM Clean-up Kit was used for the clarification of sera from patients with rheumatoid arthritis (RA) containing low, medium and high concentrations of rheumatoid factor (RF), alongside a sample of pooled normal human serum and plasma as controls. Untreated and treated samples were then used for the analysis of IL-1Ra expression in duplicate using our Firefly® human IL-1ra Multiplex Immunoassay Kit (ab208224). The concentrations of IL-1Ra were interpolated from the IL-1Ra standard curves and corrected for sample dilution. Robust quantitation of IL-1Ra was not possible in untreated RA patient sera with medium and high levels of RF due to poor Firefly particle recovery, however clarification using the Rheumatoid factor and IgM Clean-up Kit restored assay performance for the detection of reliable IL-1Ra expression, without any effect upon IL-1ra measurement in treated healthy control serum or plasma.
The Rheumatoid factor and IgM Clean-up Kit was used for the clarification of sera from a patient with rheumatoid arthritis (RA) containing medium concentrations of rheumatoid factor (RF), alongside a sample of pooled normal human serum. Untreated and treated samples were then used for the quantitation of IgM levels in duplicate using our SimpleStep® human IgM ELISA Kit (ab214568). The concentrations of IgM were interpolated from the IgM standard curves and corrected for sample dilution. Upon treatment with the Rheumatoid factor and IgM Clean-up Kit, mean IgM concentrations in normal human serum were reduced by 56% and in medium RF serum by 83%.

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