

## HeteroBlock<sup>®</sup> Usage

1. Anderson, Amy E., et al. (2016), IL-6-driven STAT signaling in circulating CD4+ lymphocytes is a marker for early anticitrullinated peptide antibody-negative rheumatoid arthritis. *Ann Rheum Dis*, 75: 466-473. doi:10.1136/annrheumdis-2014-205850

**Keywords:** Soluble gp130 (sgp130) and IL-23 measurements were made using a Quantikine ELISA kit (R&D systems, Minneapolis, ..., USA). Manufacturer guidance was adhered to, but a cocktail of non-human sera (**Heteroblock**, Bozeman, ..., USA) was added to each assay at an optimized final concentration of **32 µg/mL** to correct for potential assay interference by heterophilic antibodies present in sera.

2. Nagafuchi, Yasuo, et al. (2016), Immunophenotyping of rheumatoid arthritis reveals a linkage between HLA-DRB1 genotype, CXCR4 expression on memory CD4+ T cell, and disease activity. *Sci Rep, Nature*, 6: 29338. doi: 10.1038/srep29338

**Keywords:** To reduce false amplification by heterophilic antibodies, **HeteroBlock (Omega Biologicals)** was added to all serum samples to achieve a final concentration of **150 µg/ml**.

3. Dahlstrom, Kristina R., et al. (2015), HPV serum antibodies as predictors of survival and disease progression in patients with HPV-positive squamous cell carcinoma of the oropharynx. *Clin Cancer Res*, 21 (12): 2861-2869. doi: 10.1158/1078-0432.CCR-14-3323

**Keywords:** The in vitro translation products were captured on magnetic microspheres (BioRad, Hercules, CA), pooled, and blocked with **HeteroBlock (Omega Biologicals, Bozeman, MT)** diluted to **2 µg/mL** into SeaBlock (Thermo Scientific, Rockford, IL). Sera were diluted 1:80 and incubated with the pooled beads and bound IgG was detected using median fluorescent intensity (MFI).

4. Kuller, Lewis H., et al. (2014). Rheumatoid Arthritis in the Women's Health Initiative: Methods and Baseline Evaluation, *Am. J. Epidemiol.* 179 (7): 917-926 first published online February 24, 2014 doi:10.1093/aje/kwu003

**Keywords:** All cytokine profiling analyses were performed using **3 µg/mL HeteroBlock (Omega Biologicals Inc., Bozeman, Montana)** in order to minimize nonspecific false elevations in cytokine readouts due to RF.

5. Andersen, M., et al. (2014). Synovial explant inflammatory mediator production corresponds to rheumatoid arthritis imaging hallmarks: a cross-sectional study. *Arthritis Res Ther*, 16(3), R107. doi:10.1186/ar4557

**Keywords:** The significance of HA interference was tested prior to analysis by adding **200 µg/ml HeteroBlock (Omega Biologicals, Bozeman, MT, USA)**, which previously has been reported to block HA interference.

6. Hughes-Austin, et al. (2013). Multiple cytokines and chemokines are associated with rheumatoid arthritis-related autoimmunity in first-degree relatives without rheumatoid arthritis: Studies of the Aetiology of Rheumatoid Arthritis (SERA). *Ann Rheum Dis*, 72 (6) 901-907. doi: 10.1136/annrheumdis-2012-201505

**Keywords:** RF-IgM may cause false-positive results in this bead-based assay by crosslinking the capture and detection antibodies, **Heteroblock** reagent (**Omega Biologicals Inc., Bozeman, Montana, USA**) was used in all samples (**3 µg/ml** of serum) to minimise the effect of RF. Due to recent reports describing use of higher **Heteroblock** concentrations, validation studies using a higher **Heteroblock** concentration (**50 µg/ml** of serum) were performed, and produced similar results to those reported herein.

7. Matthey, D. L., Nixon, N. B., & Dawes, P. T. (2012). Association of circulating levels of MMP-8 with mortality from respiratory disease in patients with rheumatoid arthritis. *Arthritis Res Ther*, 14(5), R204. doi:10.1186/ar4042

**Keywords:** Sample preparation and assay procedure were followed according to the manufacturer's recommendation. **Heteroblock (Omega Biologicals, Bozeman, MT, USA)** was added at a concentration of **150 µg/ml** into the sample diluent prior to assay, to block any non-specific binding to RF.

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8. Law, S. C., et al. (2012). T-cell autoreactivity to citrullinated autoantigenic peptides in rheumatoid arthritis patients carrying HLA-DRB1 shared epitope alleles. *Arthritis Res Ther*, 14(3), R118. doi:10.1186/ar3848

**Keywords:** Where RA serum was used in cytokine production assays, **150 µg/ml HeteroBlock (Omega Biologicals Bozeman, MT, USA)** were added during BD Cytometric Bead Array measurement. ... More-over, there was no difference in cytokine production when comparing assays carried out in the presence of 10% healthy or autologous or allogeneic RA donor serum, provided **HeteroBlock** was added when rheumatoid factor titres were > 100 U/ml (data not shown) to prevent rheumatoid factor from binding capture and detection antibodies in ELISA reactions.

9. Todd, D. J., et al. (2011), Erroneous augmentation of multiplex assay measurements in patients with rheumatoid arthritis due to heterophilic binding by serum rheumatoid factor. *Arthritis Rheum*, 63: 894–903. doi: 10.1002/art.30213

**Abstract:** Serum rheumatoid factor (RF) and other heterophilic antibodies potentially interfere with antibody-based immunoassays by nonspecifically binding detection reagents. The purpose of this study was to assess whether these factors confound multiplex-based immunoassays, which are used with increasing frequency to measure cytokine and chemokine analytes in patients with rheumatoid arthritis (RA). We performed multiplex immunoassays using different platforms to measure analyte concentrations in RA patient samples. Signal amplification by heterophilic antibodies was blocked effectively by **HeteroBlock (≥150 µg/ml)**. In 35 RA patients, multiplex signals for 14 of 22 analytes were amplified erroneously in unblocked samples as compared to blocked samples (some >100-fold), but only in patients with high-titer RF (P < 0.002).

10. Song, J. J., et al. (2011). Plasma carboxypeptidase B downregulates inflammatory responses in autoimmune arthritis. *J Clin Invest*, 121(9), 3517–3527. doi:10.1172/JCI46387

**Keywords:** To block nonspecific cross-linking by rheumatoid factor, we preincubated synovial fluid samples with **3 µg/ml of HeteroBlock (Omega Biologicals)**.

11. Chandra, P. E., et al. (2011). Novel multiplex technology for diagnostic characterization of rheumatoid arthritis. *Arthritis Res Ther*, 13(3), R102. doi:10.1186/ar3383

**Keywords:** To prevent RF from bridging capture and detection antibodies in the immunoassays, we added **Heteroblock (Omega Biologicals, Bozeman, MT, USA)** to the sera at a final concentration of **3 µg/ml** (we have shown that this concentration of **Heteroblock** eliminates false augmentation of the readout by heterophilic antibodies).

12. Sharif, Shadi A., et al. (2009) Thrombin-activatable carboxypeptidase B cleavage of osteopontin regulates neutrophil survival and synoviocyte binding in rheumatoid arthritis. *Arthritis Rheum*, 60 (10) DOI: 10.1002/art.24814

**Keywords:** To block nonspecific crosslinking by rheumatoid factor, samples were preincubated with **3 µg/ml HeteroBlock (Omega Biologicals, Bozeman, MT)**.

13. Hueber, W., et al. (2009). Blood autoantibody and cytokine profiles predict response to anti-tumor necrosis factor therapy in rheumatoid arthritis. *Arthritis Res Ther*, 11(3), R76. doi:10.1186/ar2706

**Keywords:** Briefly, to minimize potential false-positive elevations of cytokine measurements due to rheumatoid factor and other heterophilic antibodies that can cross-link the capture and detection antibodies, **HeteroBlock<sup>®</sup>** was added to achieve a final concentration of **3 µg/ml**, as previously described in detail.

14. Hueber, W., et al. (2007). Proteomic analysis of secreted proteins in early rheumatoid arthritis: anti-citrulline autoreactivity is associated with up regulation of proinflammatory cytokines. *Ann Rheum Dis*, 66(6), 712–719. doi:10.1136/ard.2006.054924

**Keywords:** Unless stated otherwise, an additional blocking reagent optimised for sandwich immunoassays (**HeteroBlock, Omega Biologicals, Bozeman, Montana, USA**) was added to the serum sample buffer to achieve **3 µg/ml** final concentration.