HeteroBlock® Usage

1. Disanto, G., et al. (2017), Serum Neurofilament Light: A Biomarker of Neuronal Damage in Multiple Sclerosis. *Annals of Neurology*, 81 (6): 857-870. (doi: 10.1002/ana.24954)

Keywords: The assay was run on a Simoa HD-1 instrument (Quanterix) using a 2-step Assay Neat 2.0 protocol; 100µl of calibrator/sample (diluent: Tris-buffered saline [TBS], 0.1% Tween20, 1% milk powder, **400µg/ml Heteroblock** [**Omega Biologicals**, Bozeman, MT]), 25µl conjugated beads (diluent: TBS,0.1% Tween 20, 1% milk powder, **300µg/ml Heteroblock**),and 20µl of mAB 2:1 (0.1µg/ml; diluent: TBS, 0.1% Tween20, 1% milk powder, **300µg/ml Heteroblock**) were incubated for 47 cadences (1 cadence = 45 seconds).

2. Makiveuchuk, E., Ruge, T., Nilsson, S., Södergren, A., Olivecrona, G. (2017), High Concentrations of Angiopoietin-Like Protein 4 Detected in Serum from Patients with Rheumatoid Arthritis Can Be Explained by Non-Specific Antibody Reactivity. *PLoS ONE*, 12 (1): e0168922. (doi: 10.1371/journal.pone.0168922)

Keywords: To investigate the possibility that we detected non-specific reactions in the ELISA for ANGPTL4 in sera from patients with RA, we added **HeteroBlock®** to the SEC fractions. When added in high concentration, **HeteroBlock** reduced the immunoreaction for ANGPTL4 in fractions from the first peak, while the reaction in the second peak was unaffected (Table 1). In addition, **HeteroBlock®** added at **50 µg/ml** eliminated most of the high immunoreactivity for ANGPTL4 in sera from patients with RA (Table 1). From this we concluded that the high reactivity for ANGPTL4 in serum from RA patients was most likely due to a false, non-specific reaction with RF/IgM.

3. Anderson, A. 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.

4. Jones, D. S., et al. (2016), Profiling drugs for rheumatoid arthritis that inhibit synovial fibroblast activation. *Nature Chemical Biology*, 13:38-45. (doi: 10.1038/NCHEMBIO.2211)

Keywords: For analysis of cytokine profiles by Luminex, supernatants were diluted 1:3 with 1× PBS, 0.05% BSA, 0.05% Tween-20, **225 µg/mL Heteroblock** (**Omega Biologicals**; final **Heteroblock** concentration, **150 µg/mL**) and processed according to the supplier's protocol. **Heteroblock** blocks potential nonspecific signal amplification by rheumatoid factor or other heterophilic antibodies in ELISA-type assays of RA samples.

5. Dahlstom, K. 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).

6. Kuller, L. 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.

7. Hughes-Austin, J. M., 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.

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8. Bellatin, M. F., et al. (2012), Production of Autoantibodies against Citrullinated Antigen/Peptides by Human B Cells. *The Journal of Immunology*, 188 (7) 3542-3550. (doi: 10.4049/jimmunol.1100577)

Keywords: Serum levels of five cytokines (IL-2, IL-4, IFN-g, TNF-a, and GM-CSF) were measured using a human cytokine Milliplex map kit (Millipore). The assay was performed according to the protocol, and was collected using a Luminex 100 instrument (Luminex, Austin, TX). To remove potential nonspecific effects in the cytokine assay due to the presence of RF, in parallel experiments, the sera from RA patients were treated with a blocking reagent developed for this purpose (**HeteroBlock**; **Omega Biologicals**, Bozeman, MT). **HeteroBlock** was added to the serum sample to achieve a final concentration of **3 µg/ml**, a concentration reported to be optimal for this assay. A much higher concentration of this reagent has recently been used by other workers, but was not thought necessary in our experiments.

9. 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.

10. 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).

11. 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**).

12. 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: 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 ($P < 0.002$).

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®** was added to achieve a final concentration of **3 µg/ml**, as previously described in detail.

14. 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).

15. 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.