Human Guanylate Binding Protein-1
ELISA Quantitation Kit

Manual

Catalog number: 40-288-23437

For the quantitative determination of hGBP-1 levels in CSF, serum or other biological samples.
This kit is for research use only, and is not for use in diagnostic procedures.

GenWay Biotech, Inc.
6777 Nancy Ridge Drive
San Diego, CA 92121
Phone: 858.458.0866
Fax: 858.458.0833
Email: techline@genwaybio.com
http://www.genwaybio.com
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**Introduction**

Human guanylate binding protein-1 (hGBP-1) is an interferon-γ-induced GTPase with a molecular weight of 67 kDa which places it in the large GTPase family (1). hGBP-1 hydrolyzes GTP and GDP but fails to hydrolyze ATP, CTP, or UTP at a reasonable rate (1). GTPases induced by interferons, such as hGBP-1, play a significant role in the immune response to microbial and viral pathogens. Moreover, hGBP-1 regulates the inhibitory effects of inflammatory cytokines on endothelial cell proliferation, migration and invasiveness (2,3,4,5) and has been shown to be associated with the inhibition of angiogenesis and a highly significantly increased survival of patients with colorectal carcinoma (6). Furthermore, it has been shown that hGBP-1 is secreted from endothelial cells and is the first GTPase known to be secreted (7). hGBP-1 exhibits anti-viral activity against vesicular stomatitis virus and encephalomyocarditis and has been shown to be detectable in the cerebrospinal fluid of patients with bacterial meningitis (7,8).

The crystallographic structure of hGBP-1 has been determined in the absence and presence of GTP (5). The protein consists of two domains: an α-helical domain at the C-terminus and a globular domain containing the GTPase functional site at the N-terminus (5). The functional site consists of a conserved arginine-aspartate acid (RD) motif instead of the classical [(N/T)KXD] motif of classical tripartite GTPases which typically enables the binding of the other nucleotides (9,10). hGBP-1 also lacks the classical leader peptide sequence for most secreted proteins. It has been shown that hGBP-1 secretion occurs via a nonclassical pathway, independent of its GTPase activity and isoprenylation, and does not require interferon-γ-induced factors (7). The detectable presence of hGBP-1 in the CSF of bacterial meningitis patients and its association with increased survival in patients with colorectal carcinoma imparts a potentially useful clinical biomarker for the diagnostic purposes.

1. Schwemmle, M, Staeheli, P. The Interferon-induced 67-kDa guanylate-binding Protein (hGBP1) is a GTPase that converts GTP to GMP. J. Bio Chem. 1994. 269(15): 11299-11305.
ELISA Kit Validation Data

Figure 1. hGBP-1 ELISA kit validation in fresh culture medium. A) The ELISA antibodies are specific for hGBP-1-His and do not recognize hGBP-3-His, BSA, His-eGFP or the isotype coating antibody. B) Coomassie stained SDS-PAGE for the protein controls.

Figure 2. IFN-γ induction of hGBP-1. A) Western blot illustrating that hGBP-1 is induced by IFN-γ. B) IFN-γ induction of hGBP-1 is both concentration and time dependent. C) hGBP-1 is induced by IFN-γ and secreted into the media. Cell viability was verified to ensure hGBP1 levels resulted from secretion and not cell lysis.

Figure 3. hGBP-1 ELISA kit validation in human serum and as a diagnostic tool for bacterial meningitis. A) The ELISA specifically recognizes hGBP-1 and not hGBP-3, His-eGFP or BSA. B) hGBP-1 concentrations determined with ELISA kit using the CSF from control patients and patients with bacterial meningitis show elevated levels for diseased patients.

**Materials Not Provided**

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbance paper or paper towel
6. Graph paper

**Kit Contents:** (enough for one 96-well plate)

1. **WASH SOLUTION CONCENTRATE**
   a. One bottle containing 25 mL of a 20X concentrated wash solution, 0.1% Tween-20 in PBS, pH 7.4

2. **DILUENT BUFFER CONCENTRATE**
   a. One bottle containing 25 mL of a 5X concentrated diluent buffer, 50 mM Tris, 0.14 M NaCl, 1% BSA, pH 8.0

3. **DETECTION ANTIBODY**
   a. One vial containing 10 uL of polyclonal rabbit anti-GBP-1 antibody.

4. **DETECTION ENZYME**
   a. One vial containing 10 uL of Goat anti-Rabbit IgG

5. **TMB SUBSTRATE SOLUTION**
   a. Two bottles, one bottle containing 6 mL of TMB Substrate A; one bottle containing 6 mL of TMB Substrate B

6. **STOP SOLUTION**
   a. One bottle containing 12 mL of 0.3M sulfuric acid

   **WARNING:** Avoid contact with skin

7. **ANTI-HUMAN GBP-1 ELISA MICRO PLATE**
   a. Twelve removable eight (8) well micro well strips in well holder frame. Each well is coated with Rat monoclonal anti- hGBP-1 antibody

8. **RECOMBINANT GBP-1 CALIBRATOR**
   a. One vial containing 40 µL with 25 µg/mL of recombinant GBP-1 calibrator.
Reagent Preparation:

1. **WASH SOLUTION CONCENTRATE**
   a. The Wash Solution supplied is a 20X Concentrate and must be diluted 1:20 with distilled or deionized water

2. **DILUENT BUFFER CONCENTRATE**
   a. The Diluent Buffer supplied is 5X Concentrate and must be diluted 1:5 with distilled or deionized water

3. **DETECTION ANTIBODY**
   a. The required amount of detection antibody for each plate is prepared by adding 4.8 µL of Detection Antibody in 12 mL of 1X Diluent Buffer (1:2500 dilution). Mix gently and avoid bubbles.

4. **DETECTION ENZYME**
   a. The required amount of detection enzyme for each plate is prepared by adding 6 µL of Detection Enzyme in 12 mL of 1X Diluent Buffer (1:2000 dilution). Mix gently and avoid bubbles.

5. **TMB SUBSTRATE SOLUTION**
   a. Mix equal parts A and B

6. **STOP SOLUTION**
   a. Ready to use as supplied

7. **ANTI- HUMAN GBP-1 ELISA MICRO PLATE**
   a. Ready to use as supplied

8. **RECOMBINANT GBP-1 CALIBRATOR**
   a. The calibrator is supplied at a concentration of 25 µg/mL. Standards need to be prepared immediately prior to use. Mix well and avoid bubbles. The calibrator should be aliquoted and stored frozen avoiding multiple freeze-thaw cycles.

<table>
<thead>
<tr>
<th>Standard</th>
<th>ng/mL</th>
<th>Amount</th>
<th>Volume of 1x Diluent</th>
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<tbody>
<tr>
<td>1</td>
<td>1000</td>
<td>40 µL of Human CRP Calibrator</td>
<td>960 µL</td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>500 µL standard 1</td>
<td>500 µL</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>500 µL standard 2</td>
<td>500 µL</td>
</tr>
<tr>
<td>4</td>
<td>125</td>
<td>500 µL standard 3</td>
<td>500 µL</td>
</tr>
<tr>
<td>5</td>
<td>62.5</td>
<td>500 µL standard 4</td>
<td>500 µL</td>
</tr>
<tr>
<td>6</td>
<td>31.25</td>
<td>500 µL standard 5</td>
<td>500 µL</td>
</tr>
<tr>
<td>7</td>
<td>15.6</td>
<td>500 µL standard 6</td>
<td>500 µL</td>
</tr>
</tbody>
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Notes:
RANGE OF DETECTION: 15.6 – 1000 ng/mL
SHELF LIFE: The expiration date for the package material is stated on the box

1. WASH SOLUTION CONCENTRATE
   a. The 1X working solution is stable for at least 1 week after preparation. Store Wash Solution at 4-8°C or at room temperature

2. DILUENT BUFFER CONCENTRATE
   a. The 1X working solution is stable for at least 1 week after preparation. Store Wash Solution at 4-8°C.

3. DETECTION ANTIBODY
   a. Store at 4-8°C and dilute immediately prior to use. DO NOT FREEZE

4. DETECTION ENZYME
   a. Store at 4-8°C and dilute immediately prior to use.

5. TMB SUBSTRATE SOLUTION
   a. Store at 4-8°C. Mix immediately prior to use.

6. STOP SOLUTION
   a. Store at 4-8°C.

7. ANTI- HUMAN GBP-1 ELISA MICRO PLATE
   a. Store at 4-8°C.

8. RECOMBINANT GBP-1 CALIBRATOR
   a. The Calibrator should be aliquoted and stored frozen (avoid multiple freeze-thaw cycles). The working solutions should be prepared immediately prior to use.

Assay Condition: The kit performance has been optimized for the protocol and materials listed below using standard dilutions of human GBP-1 in the 15.6 - 1000 ng/mL range. **The operator must determine appropriate dilutions of reagents for alternative assay conditions.** ELISA assay reactivity is sensitive to variations in operator, pipetting and washing techniques, incubation time, temperature, composition of reagents, and other experimental variables. Assay optimization may be required to generate the standard curve and fit the samples in the specified detection range.

Country of Origin: United States of America

Assay Use: For Research Use Only (RUO). Not for diagnostic or therapeutic use in humans or animals. Not for animal or human consumption.
**hGBP-1 Quantitative ELISA Protocol**

*Step-by-Step Method (Perform all steps at room temperature)*

1. **Standards, Controls, and Samples**
   A. Dilute the standards in 1X Diluent buffer according to the table on page 8. Perform serial dilutions at a 1:2 (one part sample, one part diluent) ratio to achieve the following concentrations: 1000, 500, 250, 125, 62.5, 31.25, 15.6 and 0 ng/mL. The detection limit is 15.6 ng/mL. Therefore, if samples are below 31.25 ng/mL, you may wish to adjust standards to provide a standard between 31.25 – 15.6 ng/mL.
   B. Dilute the samples, based on the expected concentration of the analyte, to fall within the concentration range of the standards. (CSF should be diluted 1:8, other dilutions for serum or conditioned media should be determined)
   C. Transfer 100 µl of standard, control or sample to assigned wells. The standards and each sample should be measured in duplicate or triplicate as space permits.
   D. Incubate plate for 2 hours at room temperature.
   E. After incubation, decant samples and standards. Fill each well with appropriately diluted Wash Solution and aspirate. Repeat 4-5 times.

2. **Detection Antibody**
   A. Dilute the detection antibody with 1X Diluent buffer (1:2500).
   B. Transfer 100 µL to each well.
   C. Incubate 2 hours at room temperature.
   D. After incubation, remove detection antibody solution and wash each well 5 times as in Step 1.E.

5. **HRP Conjugated Antibody**
   A. Dilute the detection enzyme with 1X dilution buffer (1:2000).
   B. Transfer 100 µL to each well.
   C. Incubate 1 hour.
   D. After incubation, remove detection enzyme solution and wash each well 4 times as in Step 1.E.

6. **Enzyme Substrate Reaction**
   A. Prepare the TMB Substrate solution by mixing equal parts, TMB Solution A and TMB Solution B.
   B. Transfer 100 µL of TMB substrate solution to each well.
   C. Incubate for 5-30 minutes
   D. Stop the reaction, by adding 100 µL of the Stop Solution to each well.

7. **Plate Reading**
   A. Using a microplate reader, read the plate at the wavelength that is appropriate for the substrate used (450 nm for TMB).
Calculation of Results

1. Average the duplicate or triplicate readings from each standard, control, and sample.
2. Subtract the zero reading from each averaged value above.
3. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. Other curve fits may also be used.
4. A standard curve should be generated for each set of samples (see examples below)
5. Determine the concentration of unknowns from the standard curve using absorbance readings for the samples.

Technical Hints

1. Change pipette tips between each addition of standard, sample and reagents to avoid cross-contamination.
2. Standards and samples should be pipetted to the bottom of the wells; all other reagents should be added to the side of the wells to avoid contamination. When pipetting, avoid bubbles and foam.
3. Make sure that all buffers are not contaminated or expired. When troubleshooting ELISA results, it is recommended to prepare new buffers.
4. Do not add sodium azide to the buffers.
5. Sample and Conjugate dilutions should be prepared shortly before use.
6. When preparing serial dilutions, wipe excess antibody/analyte from pipette tips to ensure accurate dilutions.
7. Incubation time of the HRP Substrate will depend on the formulation used and the intensity of the color change. The high standard should have an O.D. reading of about 2.0 and the low standard should have an O.D. reading above background.
8. The Stopping solution should be added to the wells in the same order as the HRP Substrate to ensure uniform chromogen reaction.
Troubleshooting

1. **Problem: Low absorbance**
   - Incorrect dilutions or pipetting errors.
   - Improper incubation times.
   - Improper preparation of the TMB substrate.
   - Wrong filter on microtiter reader. The wavelength should be 450 nm for TMB, 490 nm for OPD, or 405 nm for ABTS.
   - Kit materials or reagents are contaminated or expired.
   - Incorrect reagents used.

2. **Problem: High Absorbance**
   - Cross contamination from other samples or positive control.
   - Incorrect dilutions or pipetting errors.
   - Improper washing.
   - Wrong filter on microtiter reader.
   - Contaminated buffers or enzyme substrate.
   - Improper incubation times.
   - Kit materials or reagents are contaminated or expired.

3. **Problem: Poor Duplicates**
   - Inadequate preparation (e.g., mixing) of specimens.
   - Incorrect dilutions or pipetting errors.
   - Technical errors.
   - Inconsistency in following ELISA protocol.
   - Inefficient washing.

4. **Problem: All wells are positive**
   - Contaminated buffers or enzyme substrate.
   - Incorrect dilutions or pipetting errors.
   - Kit materials or reagents are contaminated or expired.
   - Inefficient washing.

5. **Problem: All wells are negative**
   - Procedure not followed correctly.
   - Contaminated buffers or enzyme substrate.
   - Contaminated conjugate.
   - Kit materials or reagents are contaminated or expired.

TECHNICAL SUPPORT:

**GenWay Biotech, Inc.**
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Fax: 858.458.0833
Email: techline@genwaybio.com
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