# ELISA PRODUCT INFORMATION & MANUAL

# Human Complement Component C2 ELISA Kit (Colorimetric)

## *NBP3-20180*

***Sample insert for reference use only***

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

# BACKGROUND

Complement component C2 is part of the classical complement pathway which plays a major role in innate immunity against infection. C2 is a glycoprotein synthesized in liver hepatocytes and several other cell types in extrahepatic tissues. This pathway is triggered by a multimolecular complex C1, and subsequently the single-chain form of C2 is cleaved into two chains referred to C2a and C2b by activated C1. The second component of complement (C2) is a multi-domain serine protease that provides catalytic activity for the C3 and C5 convertases of the classical and lectin pathways of human complement. C4b and C2 was investigated by surface plasmon resonance. C2a containing a serine protease domain combines with complement component C4b to form the C3 convertase C4b2a which is responsible for C3 activation, and leads to the stimulation of adaptive immune responses via Lectin pathway. C2 bound to C4b is cleaved by classical (C1s) or lectin (MASP2) proteases to produce C4bC2a. C2 has the same serine protease domain as C4bC2a but in an inactive zymogen-like conformation, requiring cofactor-induced conformational change for activity. Deficiency of C2 (C2D) is the most common genetic deficiency of the complement system, and two types of C2D have been recognized in the context of specific MHC haplotypes. C2D in human is reported to increase susceptibility to infection, and is associated with certain autoimmune diseases, such as rheumatological disorders.

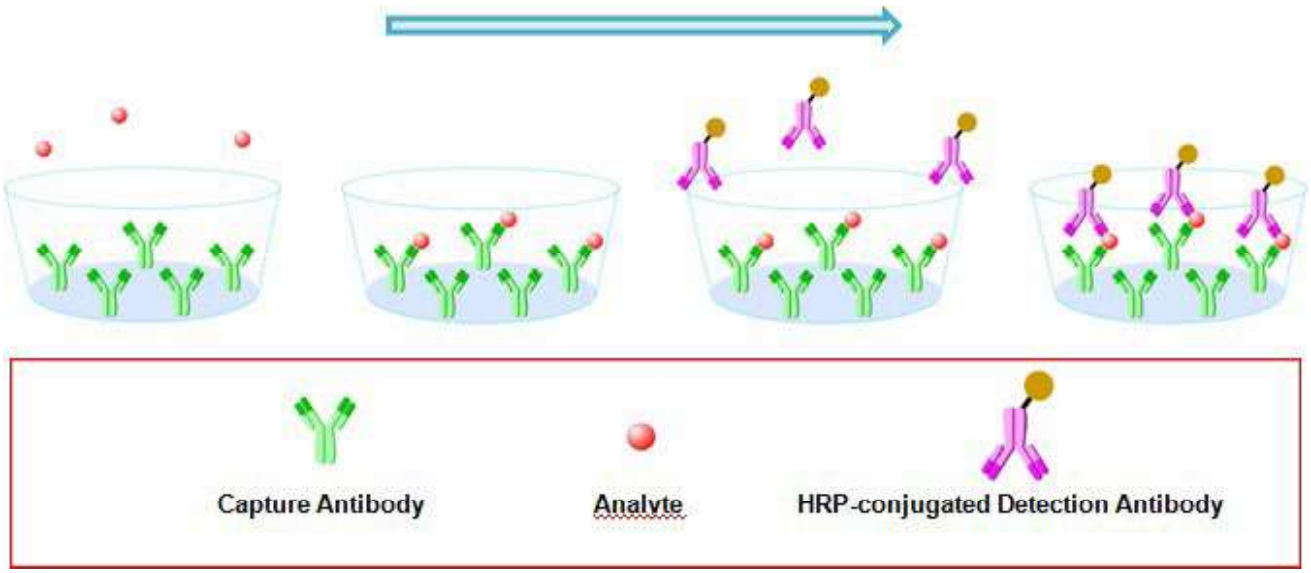
# INTENDED USE

For the quantitative determination of Human Complement Component C2 concentration in serum, or cell culture supernates.

**The use of this kit for other sample types need be validated by the end user due to the complexity of natural targets and unpredictable interference.**

## PRINCIPLE OF THE ASSAY

The principle of this ELISA kit is based on the solid phase sandwich enzyme immunoassay technique. A monoclonal antibody specific for Human Complement Component C2 has been pre-coated onto well plate strips. Standards and samples are added to the wells and Human Complement Component C2 present in the sample is bound by the immobilized antibody. After incubation the wells are washed and a horseradish peroxidase conjugated anti-Human Complement Component C2 antibody is added, producing an antibody-antigen-antibody "sandwich complex". Following a wash to remove any unbound antibody a TMB substrate solution is loaded and color develops in proportion to the amount of Human Complement Component C2 bound. The reaction is stopped by the addition of a stop solution and the intensity of the color can be measured at 450 nm (See schematics below).



## MATERIALS PROVIDED

Human Complement Component C2 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with rabbit monoclonal antibody against Human Complement Component C2.

Human Complement Component C2 Detection Antibody - 0.1 mg/mL of mouse monoclonal antibody against Human Complement Component C2 conjugated to horseradish peroxidase (HRP) with preservatives.

Human Complement Component C2 Standard - Recombinant Human Complement Component C2 in a buffer with preservatives, lyophilized. The amount of standard is lot specific and indicated on the label of standard vial.

Wash Buffer Concentrate - 25 mL of a 20-fold concentrated solution of buffered surfactant with preservatives.

Dilution Buffer Concentrate - 8 mL of a 20-fold concentrated dilution buffer with preservatives.

Color Reagent A - 13 mL of stabilized hydrogen peroxide.

Color Reagent B - 13 mL of stabilized chromogen (tetramethylbenzidine).

Stop Solution - 8 mL of 2 N sulfuric acid.

# STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8℃ and the kit is stable for 6 months upon receipt.</td></tr>
<tr><td rowspan="7">Opened/ Reconstituted Reagents</td><td>Diluted Wash Buffer</td><td rowspan="2">Stored for up to 1 week at 2 - 8℃</td></tr>
<tr><td>Diluted Dilution Buffer</td></tr>
<tr><td>Conjugate</td><td rowspan="3">Stored for up to 1 month at 2 - 8℃</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td><td rowspan="2">After reconstitution, store for up to 1 month at -80℃.<br>The reconstituted standards should be aliquoted and<br>avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Standard</td></tr>
<tr><td>Microplate Wells</td><td>Return unused strips to the foil pouch containing the desiccant pack and reseal along entire edge of zip-seal.<br>Stored for up to 1 month at 2 - 8℃</td></tr>
</table>

## OTHER SUPPLIES REQUIRED

·Microplate reader capable of measuring absorbance at 450 nm

·Pipettes and pipette tips

·Deionized or distilled water

·Multi -channel pipette, squirt bottle, manifold dispenser, or automated microplate washer

·500 mL graduated cylinder

·Tubes for standard dilution

·Well plate cover or seals

## PRECAUTIONS

1. This kit is **for research use only** and is not for use in diagnostic or therapeutic procedures.

2. The kit should not be used beyond the expiration date.

3. Do not mix reagents from different lots.

4. The kit is designed and tested to detect the specific targets and samples shown in the manual. The use of this kit for other purpose should be verified carefully by the end user.

## SAFETY INSTRUCTIONS

5. The Stop Solution provided with this kit is an acid solution. Take care when using the reagent to avoid the risk.

6. All biological materials should be handled and discarded as potentially hazardous following local laws and regulations.

7. Personal protective equipment such as lab coats, gloves, surgical masks and goggles are necessary in experiments for safety reasons.

## TECHINICAL TIPS

8. Bring all reagents and samples to room temperature before use.

9. Samples should be thawed completely and mixed well prior to analysis. Avoid repeated freeze-thaw cycles of frozen samples.

10. A standard curve should be generated for each set of sample assayed. DO NOT USE the standard curves from other plates or other days.

11. Use a new disposable reagent reservoir and new disposable pipette tips for each transfer to avoid cross-contamination.

12. Read the absorbance of each well within 20 minutes after adding the stop solution.

## SAMPLE COLLECTION AND STORAGE

**Serum** - Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20℃ or lower temperature. **Avoid repeated freeze -thaw cycles.**

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20℃ or lower temperature. **Avoid repeated freeze-thaw cycles. If the use of original supernate sample or low dilution (<5 fold) are necessary due to the expected low concentration of antigen supernates need be adjust to neutral pH condition before assay.**

**Note:**

The sample should be diluted to within the working range of the assay in 1× dilution buffer. The exact dilution must be determined based on the concentration of specific target in individual samples.

## REAGENT PREPARATION

**Bring all reagents to room temperature before use. If crystals have formed in buffer solution, warm to room temperature and mix gently until the crystals have completely dissolved.**

Wash Buffer - Prepare 1× wash buffer by adding 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 400 mL of Wash Buffer.

Dilution Buffer - Prepare 1× dilution buffer by adding 5 mL of Dilution Buffer Concentrate to deionized or distilled water to prepare 100 mL of Dilution Buffer.

Detection Antibody - Centrifuge at 10,000 x g for 20 seconds. Dilute to **work concentration** of 1 μg/mL in Dilution Buffer before use.

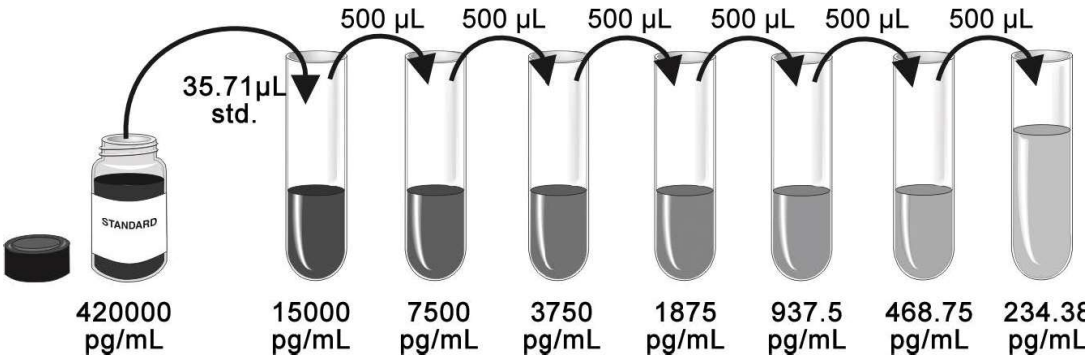
Substrate Solution **-** Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 μL of the resultant mixture is required per well. **Take care not to contaminate the Color Reagent. If the mixed color reagent is blue. DO NOT USE.**

Human Complement Component C2 Standard - Reconstitute the Human Complement Component C2 Standard with 1 mL of Dilution Buffer to make stock solution. Shake the vial gently until the lyophilized powder totally dissolved (**Do not turn the vial upside down**). Mix the standard to ensure complete reconstitution prior to making dilutions.

Prepare serially diluted standards as described in the following step:

Pipette 1000 µL of Dilution Buffer into the 15000 pg/mL tube. Pipette 500 µL of Dilution Buffer into the remaining tubes. Use the stock solution to produce a dilution series as the following figure. Mix each tube thoroughly before the next transfer. The 15000 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL). **Ensures each assay has a standard curve. DO NOT USE the standard curve on other plates or other days.**

The following graph is only for demonstration purposes. The concentration of stock solution is lot specific and need be calculated with the actual amount of standard labeled on the standard vial.



# ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.**

1. Prepare all reagents, working standards, and samples as directed in the previous sections.

2. Remove unused microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

3. Wash each well three times with Wash Buffer (300 µL/well) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. **Complete removal of liquid at each step is essential to good performance. Remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.**

4. Add 100 µL of each serially diluted protein standard or test sample per well including a zero standard. **Ensure reagent addition is uninterrupted and completed within 15 minutes.** Cover/seal the plate and incubate for 2 hours at room temperature.

5. Repeat the aspiration/wash as in Step 3.

6. Add 100 µL of Detection Antibody in working concentration to each well. Cover/seal the plate and incubate for 1 hour at room temperature.

7. Repeat the aspiration/wash as in Step 3.

8. Add 200 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature**. Protect from light.**

9. Add 50 µL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

10. **Determine the optical density of each well within 20 minutes**, using a microplate reader set to 450 nm.

# CALCULATION OF RESULTS

**If samples generate values higher than the highest standard, dilute the samples and repeat the assay.**

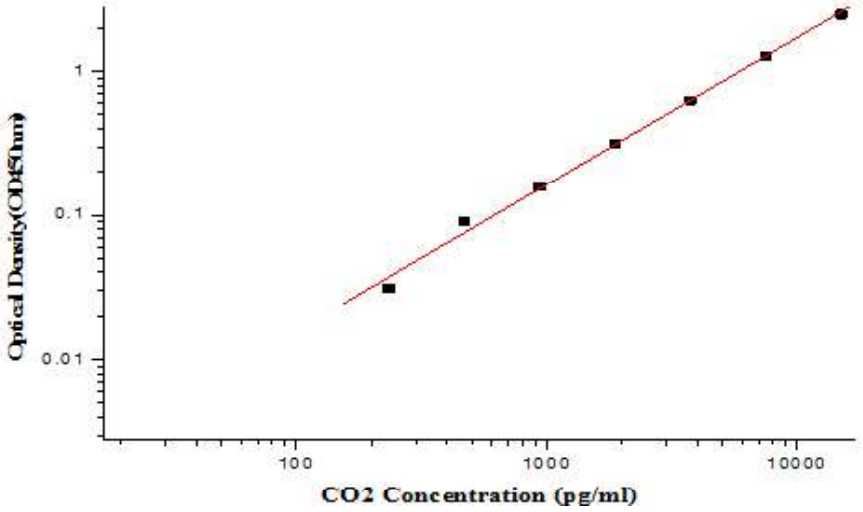
Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (O.D.).

Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. Most graphing software can help make the curve and a four parameter logistic (4-PL) usually provide the best fit, though other equations (e.g. linear, log/log) can also be tried to see which provides the most accurate. Extrapolate the target protein concentrations for unknown samples from the standard curve plotted.

# TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.

| Concentration ( pg/mL) | Zero standard subtracted OD |
|---|---|
| 0 | 0 |
| 234.375 | 0. 031 |
| 468.75 | 0. 091 |
| 937.5 | 0. 159 |
| 1875 | 0. 315 |
| 3750 | 0. 630 |
| 7500 | 1. 278 |
| 15000 | 2. 492 |



# PRECISION

**Intra-assay Precision (**Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

**Inter-assay Precision (**Precision between assays)

Three samples of known concentration were tested in five separate assays to assess inter-assay precision.

| | **Intra -assay Precision** | | | **Inter -assay Precision** | | |
|---|---|---|---|---|---|---|
| Sample | 1 | 2 | 3 | 1 | 2 | 3 |
| N | 20 | 20 | 20 | 5 | 5 | 5 |
| Mean (pg/mL) | 2165 | 4176 | 7840 | 1994 | 4188 | 8025 |
| SD | 114.79 | 151.29 | 212.45 | 195.35 | 306.31 | 742.76 |
| CV (%) | 5.3% | 3.6% | 2.7% | 9.8% | 7.3% | 9.3% |

# RECOVERY

The recovery of Human Complement Component C2 spiked to different levels throughout the range of the assay in related matrices was evaluated.

| **Sample** | **Average % Recovery** | **Range** |
|---|---|---|
| Cell culture supernates (n=3) | 102 | 97 -107% |
| Serum (n=3) | 98 | 88-105% |

# LINEARITY

| | | **Serum** | **Cell culture supernates** |
|---|---|---|---|
| 1:2 | recovery of detected | 92% | 101% |
| 1:4 | recovery of detected | 103% | 99% |
| 1:8 | recovery of detected | 105% | 97% |
| 1:16 | recovery of detected | 104% | 81% |

## SENSITIVITY

The minimum detectable dose (MDD) of Human Complement Component C2 is typically less than 110.77 pg/mL. The MDD was determined by adding three standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

## CALIBRATION

This immunoassay is calibrated against a highly purified HEK 293-expressed recombinant Human Complement Component C2.

## SAMPLE VALUES

The average concentration of C02 in 10 normal human serum is 47.86 +/- 14.60 µg/mL ranging from 21.43 to 67.86 µg/mL. HepG2 cells (0.2 x 10E5 cells/mL) were cultured 3 days. An aliquot of the cell culture supernate was removed, assayed for levels of natural CO2, and measured 252.51 ng/mL.

## SPECIFICITY

This assay recognizes both recombinant and natural Human CO2. Human C1S and C5a were prepared at 300 ng/mL in dilution buffer and assayed for cross-reactivity. No cross-reactivity was observed. The factors listed below were prepared at 50 ng/mL in dilution buffer and assayed for cross-reactivity. No cross-reactivity was observed.

| **Recombinant human** | | | |
|---|---|---|---|
| IL1 | IL2 | IL33 | IL10 |
| IL8 | PDGF | TNF-α | IL4 |
| IL6 | IFN-γ | VEGF | MIP-1β |
| TIMP1 | TIMP2 | GM-CSF | SCF |

# TROUBLE SHOOTING

| **Problems** | Possible Sources | Solutions |
|---|---|---|
| **No signal** | Incorrect or no Detection Antibody was added | Add appropriate Detection Antibody and continue |
| | Substrate solution was not added | Add substrate solution and continue |
| | Incorrect storage condition | Check if the kit is stored at recommended condition and used before expiration date |
| **Poor Standard Curve** | Standard was incompletely reconstituted or was inappropriately stored | Aliquot reconstituted standard and store at -80 ℃. The reconstituted standards should be aliquoted and avoid repeated freeze-thaw cycles. |
| | Imprecise / inaccurate pipetting | Check / calibrate pipettes |
| | Incubations done at inappropriate temperature, timing or agitation | Follow the general ELISA protocol |
| | Background wells were contaminated | Avoid cross contamination by using the sealer appropriately |
| **Poor detection value** | The concentration of antigen in samples was too low | Enriching samples to increase the concentration of antigen |
| | Samples were ineffective | Check if the samples are stored at cold environment. Detect samples in timely manner |
| **High Background** | Insufficient washes | Use multichannel pipettes without touching the reagents on the plate |
| | | Increase cycles of washes and soaking time between washes |
| | Color Reagent should be clear and colorless prior to addition to wells | Color Reagent should be clear and colorless prior to addition to wells |
| | Use clean tubes and pipettes tips | Use clean plates, tubes and pipettes tips |
| **Non-specificity** | Samples were contaminated | Avoid cross contamination of samples |
| | The concentration of samples was too high | Try higher dilution rate of samples |