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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The Human IgM Chemiluminescent ELISA Kit is designed to detect and quantify protein levels of human IgM pentamers captured by pre-matched antibody pairs. This assay kit comes in a convenient 96-well format, with a pre-coated plate with anti-IgM-specific antibody, enough recombinant purified IgM standard, and detection reagents for 100 wells.

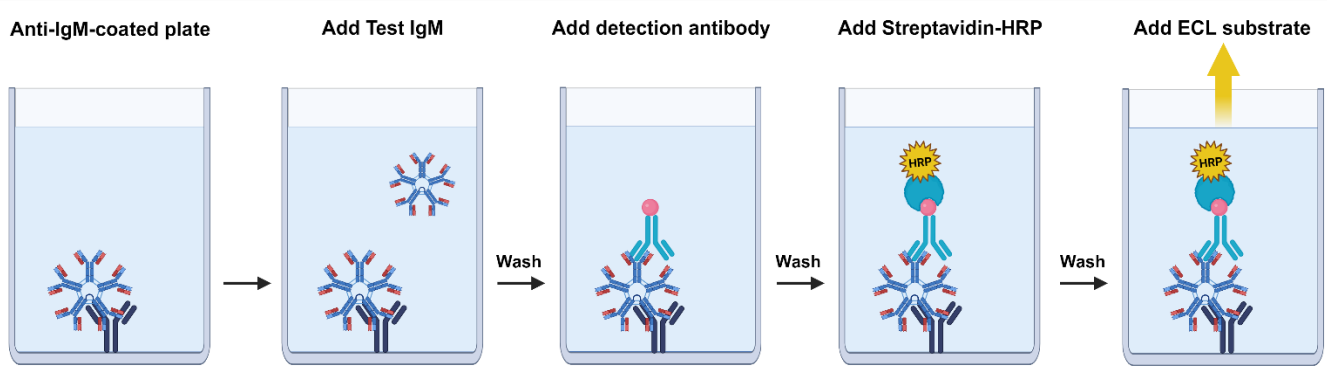

Figure 1: Human IgM Chemiluminescent ELISA Kit assay principle.

Background

IgM (immunoglobulin M), also known as macro-immunoglobulin, is an immunoglobulin isotype found in vertebrates in response to an initial exposure to an antigen. It is produced first in B cells as a membrane protein and later in plasma cells as a secreted pentamer (5 monomers adjoined by another protein called the J chain) or hexamer. IgM is a potent inhibitor of pathogens and trigger complement responses. IgM is also involved in the recognition and targeting of self-antigens or neo-epitopes for cell removal, as in the case of the recognition of phosphorylcholine, single and double stranded RNA, amongst others. In contrast to IgG, IgM does not bind to Fc gamma receptors and cannot trigger ADCC (antibody-dependent cellular cytotoxicity). IgM is being tested as therapeutic agent for the treatment of sepsis, cancer, GvHD (graft versus host disease) and MS (multiple sclerosis). Further studies are necessary to access the full potential of using IgM in the development of auto-inflammatory and cancer treatments.

Applications

Quantify human IgM in cell culture supernatants.

Supplied Materials

Catalog #	Name	Amount	Storage
	96-well Anti-IgM Pre-Coated Plate	1	+4°C
82739	Anti-IgM Detection Antibody	6 µl	-80°C
82724	Streptavidin HRP	6 µl	-80°C
79743	Blocking Buffer 3	25 ml	+4°C
82738	IgM Standard*	5 µg	-80°C
79670	ELISA ECL Substrate A	6 ml	Room Temp
	ELISA ECL Substrate B	6 ml	Room Temp
	Adhesive plate seal	1	Room Temp

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- Test Samples
- PBST Buffer (1x PBS with 0.05% Tween-20)
- Diluent Solution (e.g. cell culture medium like DMEM (Dulbecco's Modified Eagle Medium))
- Microplate reader capable of reading luminescence
- Adjustable micropipettor and sterile tips
- Orbital shaker

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

The Human IgM Chemiluminescent ELISA Kit is compatible with up to 1% final DMSO concentration.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include “Negative Control”, “IgM Standard”, and “Test Sample” conditions.
- We recommend maintaining the diluted antibody on ice during use.
- Variation in sample collection, processing and storage may cause differences in sample assay results.

- We recommend using IgM Standard (#82738) as internal control. If not running a full standard curve, we recommend running the IgM standard at 0.1X, 1X and 10X the EC_{50} value shown in the validation data below.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend adding protease inhibitors (#82199) to samples and store samples at -80°C to avoid loss of bioactive human IgM.
- Samples containing a visible precipitate must be clarified prior to use in the assay.
- Avoid repeated freeze-thaw cycles. The frozen sample should be thawed on ice and mixed gently.
- The linear range of the assay is: 0-400 ng/ml.

Step 1: IgM Binding

1. Rehydrate the plate by adding 200 µl of PBST to every well.

2. Incubate 15 minutes at Room Temperature (RT).

3. Remove the PBST and tap the plate onto clean paper towels to remove all liquid.

4. Thaw the IgM Standard on ice. Briefly spin the tube to recover the full content of the tube.

5. Dilute the IgM Standard to 8 ng/µl (50 µl/well) in the same Diluent Solution that was used for sample preparation. This will correspond to the highest value on the standard curve.

   Note: It is recommended to use the same buffer or medium as the Diluent Solution as the one used in the preparation of the "Test Sample". For example, if the uptake and recycling of human IgM antibodies by a pool of cells is analyzed, the same cell medium should be used as Diluent Solution to prepare an IgM standard. Alternatively, PBS or PP-02 Buffer (BPS Bioscience, sold separately) can be used.

6. Prepare a serial dilution (1:3 recommended) of the diluted IgM Standard using the preferred diluent solution (50 µl/well).

7. Add 50 µl of Diluent Solution to “Negative Control” wells.

8. Add 50 µl of IgM Standard dilutions to wells labeled “IgM Standard”.

9. Add 50 µl of each test sample to the wells labeled "Test Sample”.

10. Incubate the plate at RT with slow agitation for 1 hour.

11. Wash the plate three times with 200 µl of PBST Buffer per well.

Step 2: Detection

1. Dilute Anti-IgM Detection Antibody 1000-fold in Blocking Buffer 3 (50 µl/well).

2. Add 50 µl to each well.

3. Incubate at RT with shaking for 45 minutes to 1 hour.
4. Wash plate three times with 200 µl of PBST Buffer per well.
5. Dilute Streptavidin HRP 1000-fold in Blocking Buffer 3 (50 µl/well).
6. Add 50 µl to each well.
7. Incubate at RT with shaking for 30 minutes.
8. Wash plate three times with 200 µl of PBST Buffer per well.
9. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/ well).
10. Add 100 µl of mix to every well.
11. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.
12. The "Negative Control" value should be subtracted from all other values.
13. If applicable generate a standard curve of luminescence versus IgM standard concentrations and determine concentration of the "Test sample". For detailed information regarding standard curve and determination of the "Test sample" concentration refer to https://bpsbioscience.com/assay-kits-faq.

Reading Chemiluminescence

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

Example Results

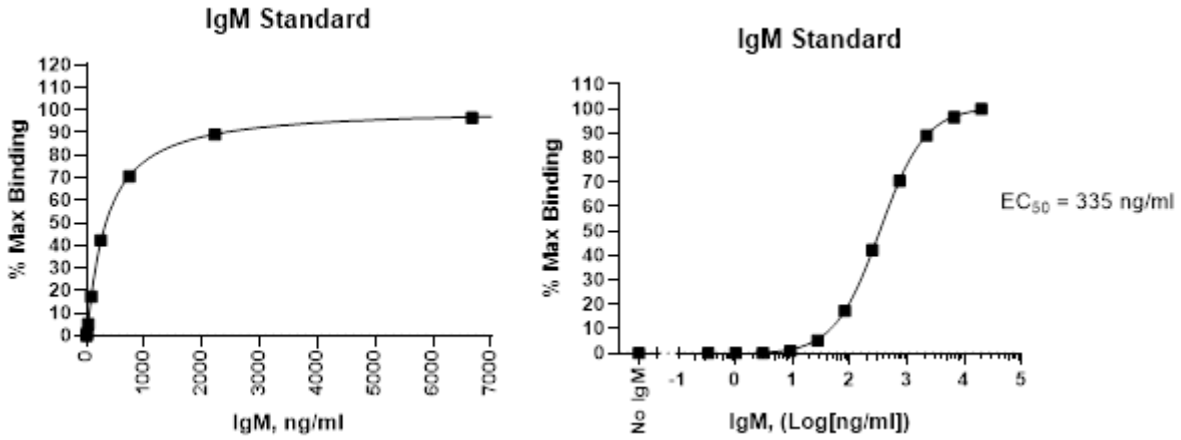

Figure 2. Example of IgM standard curves.
Various amounts of the IgM standard were run in duplicate.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

References

Keyt B., *et al.*, 2020 *Antibodies (Basel)* 9(4): 53.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
Human IgM Colorimetric ELISA Kit	82737	96 reactions
FcRn: IgM Recycling HMEC-1 Cell Pool	82163	2 vials
FcRn Recycling Wash Buffer (pH7-7.4)	82208	75 ml
FcRn Recycling Dilution Buffer (pH6)	82209	15 ml
FcRn (FCGRT/B2M) Blocker	101468	50 µg/ 100 µg
IgM4, Fc (Human) HiP™ Recombinant	71457	200 µg
Anti-Human IgM, Unconjugated Antibody	100736	100 µg/ 500 µg