INTENDED USE

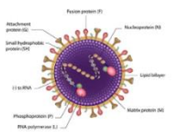
Human Anti- Respiratory Syncytial Virus Fusion Protein (RSV-F) IgM is an indirect ELISA suitable for detecting anti-RSV-F protein IgM in serum or plasma in vaccinated samples. Other biological fluids, including tissue culture medium, may be validated for use.

This immunoassay is suitable for:

- Determining immune status relative to non-immune controls
- Assessing efficacy of vaccines, including dosage, adjuvantcy, route of immunization, and timing
- Qualifying and/or standardizing vaccine batches/protocols

The kit contains no virus or virus derived antigens. For research use only (RUO). Not for diagnosis, cure, or prevention of the disease.

GENERAL INFORMATION

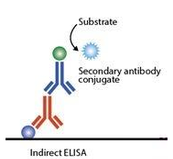


Respiratory Syncytial Virus (RSV) is a leading cause of severe respiratory infection in infants and children. RSV is a respiratory pathogen that infects **human** and **chimpanzees**. It is the most common pathogen leading to hospitalization in young children up to the age of 5. RSV Infection may result from either direct or indirect contact with oral or nasal secretions from an infected person. Specific **diagnostic tests** for confirming RSV infection include Culture, Antigen-revealing techniques, Polymerase chain reaction (PCR) assay, and serology (Antigen or antibody tests by ELISA).

RSV is an enveloped, nonsegmented, negative-sense RNA virus (~15 Kb) that encodes major viral proteins (**G, F, and SH**). Matrix protein (M) and four proteins associated with the genomic RNA form the viral nucleocapsid **(N, P, L, and M2)** open reading frame 1 [M2 ORF1]). M2 ORF-2 is a second, distinct protein transcribed from the M2 gene, which has defined properties in transcriptional regulation. Finally, two proteins (**NS1 and NS2**) are nonstructural viral products that accumulate in infected cells but are present in only trace amounts in mature virions.

Therapeutics & Vaccines: High risk infants can be treated with a neutralizing, humanized antibody to RSV (**palivizumab or Synagis**®) to reduce the risk of severe RSV. **No approved vaccine** available. A formalin-inactivated vaccine (FI-RSV) showed poor immunity. Recombinant vaccine that is suitable for intranasal instillation is being tested in the chimpanzee.

PRINCIPLE OF THE TEST



The **Human Anti-RSV-F** antibody ELISA kit is based on the binding of human antibody in samples to RSV-F antigen coated on the plate, and virus antibody is detected by a specific antibody conjugated to HRP. After a washing step, substrate (TMB) is added and color (blue) is developed, which is directly proportional to the amount of antibody present in the sample. Stop Solution is added to terminate the reaction (converts blue to yellow color), and absorbance at A450nm is then measured using an ELISA reader. The presence or concentration of antibody in samples is determined relative to supplied controls or calibrators.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8ºC until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

Component	Preparation Instructions
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at 4°C for long term and ambient temp. for short term.
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample/Conjugate Diluent** and store at 2-8°C until the kit lot expires or is used up.
Anti-Human IgM-HRP Conjugate Concentrate (100x) Part: H-HuM.2a11, 0.15ml	Peroxidase conjugated anti-human IgM in buffer with detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample/Conjugate Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8°C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
RSV-F Coated Strip Plate	510301	8-well strips (12)	Coated with purified recombinant RSV-F and post-coated with stabilizers.
Anti-RSV-F Calibrators			
1 U/ml 2.5 U/ml 5 U/ml 10 U/ml	510302B 510302C 510302D 510302E	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing anti-RSV-F; in buffer with antimicrobial as stabilizers.
Anti-RSV-F Positive Control	510302-PC	0.65 ml	Serum with anti-RSV-F reactivity; [Value range on label]
Low NSB Sample Diluent Reduces non-specific binding	TBTm **Not** for HRP Conjugate dilution.	30 ml	Buffer with protein, detergents and antimicrobial as stabilizers. Use as is for sample dilution. See **Assay Design**, page 3.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Human IgM HRP Concentrate.
- Stock bottle to store diluted Wash Solution; 0.2 to 1L.
- ELISA plate reader at 450 nm wavelength and ELISA plate washer (if available)

ASSAY DESIGN AND SET-UP

Sample Collection and Handling

Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. If samples will not be assayed immediately, store refrigerated for up to a few weeks or frozen for long-term storage.

Antibody Stability & Dilution

Initial dilution of serum into **Working Sample Diluent** (WSD) is recommended to stabilize antibody activity. This enhances reproducible sampling and stabilizes the antibody activity for years, stored refrigerated or frozen. Further dilution into **Low NSB Sample Diluent** (LNSD), which provides the lowest assay background, should be at least 10 times the initial dilution and performed the same day as the assay.

Example: Initial (1/5): **10**ul serum + **40**ul WSD [or 0.1ml + 0.4ml]
Further (1/100): **10**ul initial (1/5) + **190**ul LNSD (1/100)

Assay Design

Review Interpretation of Results (p. 5-7) before proceeding:

- Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be lower than the **1 U/ml Calibrator**. This is usually 1:100 or greater dilution for human serum with normal levels of IgG and IgM.
- Run **the Anti-RSV-F Positive Control**; value range is on vial label.
- Run a Sample Diluent **Blank**. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required. Blank OD should be <0.3.
- Run a set of **Calibrators**, which validate that the assay was performed to specifications: **10 U/ml** should give a high signal (>1.5 OD); **1 U/ml** should give a low signal which can be used to discriminate at the Positive/Negative threshold (see Interpretation of Results, p. 5).

Plate Set-up

Bring all reagents to room temperature (18-30°C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 8 Calibrator wells and 2 wells for each sample control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 1-5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of calibrators, samples, and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-Human IgM HRP to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 1.

3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices and avoid ingestion or contact of any reagent with skin, eyes, or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for all reagents can be requested or obtained from the ADI website.

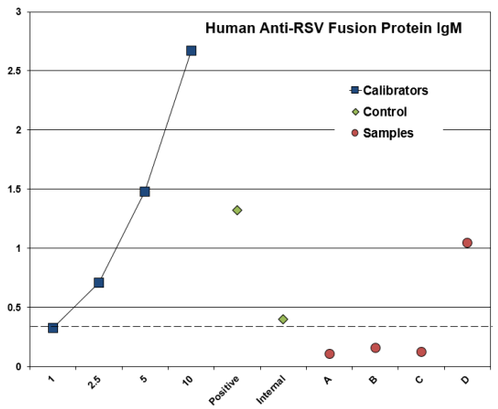
INTERPRETATION OF RESULTS

Method A. Antibody Activity Threshold Index

Compare Samples to **1 U/ml Calibrator** or **Internal Control**

= Positive/Negative Cut-off.

Example:



Results

The **sensitivity** of the assay to detect anti-RSV-F IgM, from either natural infection or vaccination, is controlled so that the **1 U/ml Calibrator** represents a threshold OD for most true positives in human serum diluted to 1:100 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of an anti-RSV-F antibody, derived from RSV-F immunization, shows the OD range of the assay; high value indicates optimal sensitivity of the assay.
1 U/ml: a 'Cut-off' line has been drawn to indicate a threshold distinguishing between **Positive/Negative**. **Note**: This is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

Positive Control – antiserum with reactivity to RSV-F; value range is on the vial label. This Control may be used to assess reproducibility and to normalize between-assay variation.

Internal Control – a true positive from an immune host that represents the investigator's experience in distinguishing low positive from negative samples (not in kit). This should be run in each assay to supplement the 1 U/ml Calibrator for Positive/Negative discrimination purposes.

Samples A,B,C,D – 3 samples (A, B, C) are negative: below the threshold; 1 sample (D) is positive: clearly above the threshold.

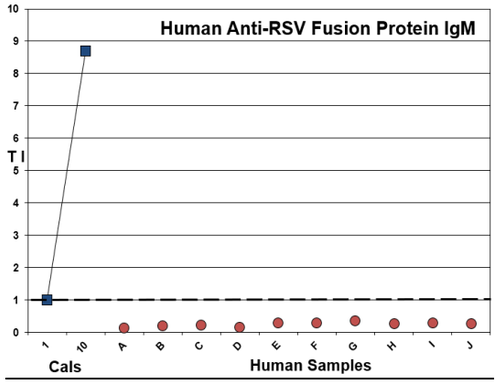
The **1 U/ml** Calibrator can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative (see p. 6):

- Divide each Sample net OD by the 1 U/ml Calibrator net OD. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

ASSAY PERFORMANCE

Example:

Human Serum IgM
A panel of sera/plasma from humans of unknown history was tested for anti-RSV-F IgM (1:100 dilution in Low NSB Diluent). **Threshold Index** was calculated using the **1** U/ml **Cal**.



Results

Human Anti-RSV-F IgM:
All ten (10) samples were negative (below 1.0 threshold) at 1:100 dilution

Notes:

1. **Positives** (of non-immunized individuals) may be due to prior encounter with the virus or non-RSV-F proteins with common epitopes, or may be an aspect of the innate immune repertoire.

2. The **sensitivity** of the assay may be adjusted by changing the sample dilutions:

   a) **increase dilution** (e.g., 1:200) to lower the signals of borderline positives to negative.

   b) **decrease dilution** (e.g., 1:50) to convert borderline samples to positive. With the latter, the values of negatives may increase, so an alternative threshold should be considered using known negatives to develop a **Positive Index (**see below) or use an **Internal Control** (Page 5).

B. Positive Index
Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a **Positive Index**. One typical method is as follows:

1. Calculate the net OD mean + 2 SD of the Control/Non-immune samples = **Positive Index**.

2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

INTERPRETATION OF RESULTS

A sample value would be **Positive** if significantly above the value of the pre-immune serum sample or a suitably determined non-immune panel or pool of samples tested at the same sample dilution.

This calculation also **quantifies** the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

Method C. Titers from Sample Dilution Curves

The titer of elevated antibody activity calculated from a dilution curve of each sample is recommended as the most accurate quantitative method. Best precision can be obtained using the following guidelines:

1. Use an OD value Index in the mid-range of the assay (2.0 – 0.5 OD); this provides the best sensitivity and reproducibility for comparing experimental groups and replicates. An arbitrary 1.0 OD is commonly used.
2. Prepare serial dilutions of each sample to provide a series that will produce signals higher and lower than the selected index. With accurate diluting, duplicates may not be required if at least 4 dilutions are run per sample.
3. A 5-fold dilution scheme is useful to efficiently cover a wide range which produces ODs both above and below 1.0 OD. The dilution scheme can be tightened to 3-fold or 2-fold for more precise comparative data.
4. The Calibrator values can be used to normalize inter-assay values.

Calculations

1. On a log scale of inverse of Sample Dilution as the x-axis, plot the OD values of the two dilutions of each positive sample having ODs above and below the OD value of the Index (arbitrary or selected Calibrator).
2. From a point-to-point line drawn between the two sample ODs, read the dilution value (x-axis) corresponding to the OD of the selected Index

= IgM Antibody Activity Units

PRODUCT SPECIFICATIONS

RSV-F Antigen



RSV-F0 protein is 574-aa. It is a single pass type 1 membrane protein found on the virus membrane. It has a signal peptide (1-25aa), F0 (26-574aa), F2 (26-136aa), F2' (26-109aa), 110-136aa (interchain peptide), and F1 (137-574aa). Extracellular domains 27-529aa. RSV-F glycoprotein F0 or RSV-F cleaved into 4 chains: F2', interchain peptide, fusion glycoprotein F2, and fusion glycoprotein F1.

Highly purified recombinant RSV-F protein (Strain A2, Extracellular domain, >95%, his-tag, ~58 kda) is used as antigen. It is highly conserved (98-100%) in various RSV strains. The Anti-Human IgM-HRP conjugate reacts specifically with Human IgM class antibodies; IgA, IgG, and IgE antibody would not be measured above background signals.

Instruction Manual No. 510-310-HRM

Human Anti- Respiratory Syncytial Virus Fusion Protein (RSV-F) IgM ELISA Kit

Cat. #510-310-HRM, 96 Tests

For the detection of RSVF IgM in serum, plasma, or other biological fluids

For in vitro research use only (RUO), not for therapeutic or diagnostic use.

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ELISA Kit Components	Amount	Part
RSV-F Antigen Coated Strip Plate	8-well strips (12)	510-301
Anti- RSV-F Positive Control	0.65 ml	510-302PC
Anti- RSV-F Calibrator 1 U/ml	0.65 ml	510-302B
Anti- RSV-F Calibrator 2.5 U/ml	0.65 ml	510-302C
Anti- RSV-F Calibrator 5 U/ml	0.65 ml	510-302D
Anti- RSV-F Calibrator 10 U/ml	0.65 ml	510-302E
Anti-Human IgM HRP Conjugate (100X)	0.15 ml	H-HuM.2a11
Sample Diluent (20x)	10 ml	SD20T
Low NSB Sample Diluent	30 ml	TBTm
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	510-310-HRM