ELISA kits available from ADI:

Human: Adiponectin (Acrp30 and gAcrp30), Albumin, Aldosterone, AFP, beta-amyloid 1-40/42, Angiogenin, Angiopoietin-2, beta-2M, BMP-7, C-peptide, CRP, Cox-2, Ferritin, PSA, fPSA, GH, IgG, IgM, IgE, IgG1, IgG4, Insulin, NSE, CA125, CA199, CA242, PAP, Resistin, SHBG, LH, FSH, TSH, T3, T4, and Steroid ELISA kits (cortisol, estradiol, testosterone, progesterone).

Monkey: IgM, IgG, IgA, IgE

Rat: Albumin, CRP, IgG, IgM, Alpha-1- Acid glycoprotein

Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG2b, IgG3, IgE, IgM, Leptin, Resistin, Acrp30, CRP, Haptoglobin, TNF-alpha
Autoimmune Antibody detection kits for ANA, ssDNA, dsDNA, Histone, Sm, RNP, SSA, SSB, Scl70, Ovalbumin, Cardiolipin, CIC

Chicken: IgG, IgM, IgY, Ovalbumin

T**urkey**: IgG

Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin

Pig: Albumin, IgG, IgM

Dog: CRP, IgG, IgM

Cat: IgG, IgM

Goat: IgG

Rabbit: CRP, IgG

Sheep: IgG

See Details at the web site or Contact ADI

Instruction Manual No. M-7630

Sheep IgM ELISA Kit

Cat # 7630, 96 Tests

For Quantitative Determination of Sheep IgM
In Serum, plasma or other biological fluids

For Research Use Only (RUO)

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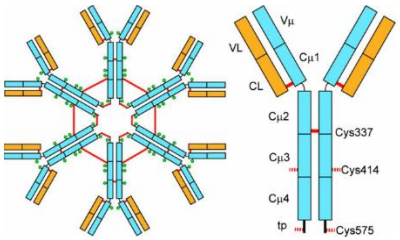
Sheep IgM ELISA KIT Cat. No. 7630, 96 tests

Kit Components, 96 tests	
Anti-Sheep IgM coated strip plate (8 wells x 12 strips), #7631	1 Plate
Sheep IgM Reference **Standard**, lyophilized in pink buffer. Reconstitute with the specified volume of distilled water and then prepare 100 ng/ml stock (see lot specific conc on the vial), #7632	3 vials
Anti-Sheep IgM HRP **Conjugate**, 11 ml, #7633	1 Bottle
Sample Diluent, 50 ml, # 7630-SD (Blue Cap)	1 Bottle
Wash Buffer (20X), 50 ml, #7630-WB (Clear Cap)	1 Bottle
TMB Substrate, 11 ml, # 7630-TM	1 Bottle
Stop solution, 11 ml, #7630-SS	1 Bottle
Instruction Manual, #M-7630	1

Intended Use:

ADI's Sheep IgM ELISA provides is a very specific and sensitive assay for Sheep IgM in serum, plasma or other biological fluids. For research use only (RUO), not for diagnosis, cure or prevention of the disease.

INTRODUCTION



Immunoglobulin M, or IgM for short, is a basic antibody that is produced by B cells. IgM is by far the physically largest antibody in the human circulatory system. It is the first antibody to appear in response to initial exposure to antigen. The spleen is the major site of specific IgM production. IgM forms polymers where multiple immunoglobulins are covalently linked together with disulfide bonds, mostly as a pentamer but also as a hexamer. IgM has a molecular mass of approximately 970 kDa (in its pentamer form). Because each monomer has two antigen binding sites, a pentameric IgM has 10 binding sites. However, IgM cannot bind 10 antigens at the same time because the large size of most antigens hinders binding to nearby sites. Because IgM is a large molecule, it cannot diffuse well, and is found in the interstitium only in very low quantities. IgM is primarily found in serum; however, because of the J chain, it is also important as a secretory immunoglobulin. Due to its polymeric nature, IgM possesses high avidity, and is particularly effective at complement activation. By itself, IgM is an ineffective opsonin; however it contributes greatly to opsonization by activating complement and causing C3b to bind to the antigen.

This ELISA kit is designed for measurement of IgM in sheep serum or plasma. The assay uses rabbit anti-sheep IgM for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated rabbit anti-sheep IgM for detection. Both capture and detection antibodies react specifically with sheep IgM. Cross-reactivity with immunoglobulins from other species has not been investigated.

PERFORMANCE CHARACTERISTICS

Detection Limit: Based on 6 replicate determinations of the zero standards, the minimum IgM concentration detectable using this assay is ~2.5 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

Specificity

Antibodies used in the kit are highly specific for IgM with no significant reactivity with sheep IgA, IgG, IgE or other serum proteins. This kit is for sheep samples only as the reactivity or detection of other species IgM has not been studies. There is usually very high cross reactivity between sheep and goat proteins.

Species Crossreactivity

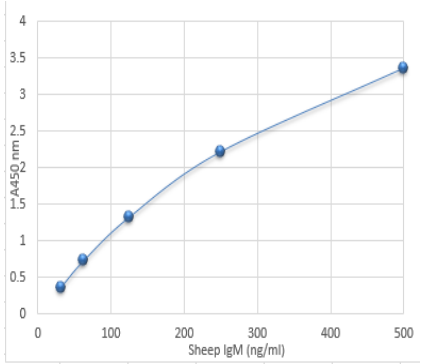
Cross-reaction of other species IgM (e.g., mouse, rat, human etc) has not been studies.

ADI provides IgG, IgM, IgA, and IgE ELISA kits For Human, Mouse, Rat, Monkey, Rabbit and Dog.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean $A_{450\ nm}$	Net mean A450
A1, A2	**Sample Diluent**		
B1, B2	**Standard A** 31.25 ng/ml		0.361
C1, C2	**Standard B** 62.5 ng/ml		0.628
D1, D2	**Standard C** 125 ng/ml		1.324
E1, E2	**Standard D** 250 ng/ml		2.218
F1, F2	**Standard E** 500 ng/ml		3.359

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



/6_ADI_ELISA

A typical assay Standard Curve (do not use this for calculating sample values)

CALCULATION OF RESULTS

Calculate the average absorbance values (A450) for each set of reference standards and samples. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis. Using the mean absorbance value for each sample, determine the corresponding concentration of IgM from the standard curve. Multiply the derived concentration by the dilution factor to determine the actual concentration of IgM in the sample. Graphing software should be used for the above steps if available. We recommend a fit using a second order polynomial equation or a single site, total and nonspecific binding equation. If the A450 values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

PRINCIPLE OF THE TEST

Sheep IgM ELISA kit is based on binding of IgM from samples to two antibodies, one immobilized on the microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (color) is directly proportional to the amount of IgM present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of IgM in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000 ul) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plates Reader.

PRECAUTIONS AND SAFETY INSTRUCTIONS

This ELISA Kit is for research use only.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separating the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera can not be immediately assayed , store frozen for up to six months. Avoid repeated freezing and thawing of samples. It is also possible to use plasma for testing.

REAGENT PREPARATION

1. **Preparation of the Standards**. Stock std vial is supplied lyophilized and upon reconstitution with the specified volume of 1x sample diluent will prepare the 500 ng/ml stock. Prepare additional standards of 500, 250, 125, 62.5 and 31.25 ng/ml by 2-fold serial dilution (dilute 100 ng/ml stock 1:2 or 250 ul stock and 250 ul 1x sample diluent and continue with the remaining standards. Stock vial can be stored at 4oC for 1 week or frozen at -20oC or below is suitable size aliquots.

2. The **Wash Buffer is a 20x stock**. Dilute the entire 50 ml with distilled or deionized water to 1 L total volume. Store at room temperature for the entire use of the kit.

Sample Preparation:

IgM is present in normal sheep serum at concentrations of ~2.5 mg/ml. In order to obtain values within range of the standard curve we suggest that samples initially be diluted 20,000 fold using the following procedure.

1. Dispense 198 ml and 497.5 ml of diluent into two tubes.

2. Pipette and mix 2.0 ml of the sample into the first tube containing 198 ml of diluent. This provides a 100 fold dilution.

3. Mix 2.5 ml of the 100 fold diluted sample with the 497.5 ml of diluent in the second tube. This provides a 20,000 fold dilution of the sample.

DILUTION OF SAMPLES

Samples containing more than 100 ng/ml IgG should be further diluted and re-tested. The results obtained should be multiplied by the appropriate dilution factor. It is possible to use normal saline or PBS for sample dilution if larger volumes of samples are taken for dilution or if more sample diluent is required.

Standard Preparation

1. Reconstitute lyophilized Reference Standard with 1 ml of distilled water and the prepare the std F using instruction on the vial and label this as stock F (500 ng/ml).. **Note**: **Stock concn is lot specific and the reconstitution volume is provided for each lot).** Store diluted stock conc at 4oC for 1-week or store frozen at 2o0-C for 6 months. .
2. Prepare additional liquid standards using the 2-fold serial dilution of 500 ng/ml stock scheme:

Sheep IgM Stock		Diluent	Final Concn	Final Volume
Concn	Volume			
Stock F	500 uL	-	**F** 500 ng/ml	500 uL
Stock E	250 uL	+ 250 uL	**E** 250 ng/ml	500 uL
Stock D	250 uL	+ 250 uL	**D** 125 ng/ml	500 uL
Stock C	250 uL	+ 250 uL	**C** 62.5 ng/ml	500 uL
Stock B	250 uL	+ 250 uL	B 31.25 ng/ml	500 uL
A (blank)	0	500	0	500

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

Label or mark the microtiter well strips to be used on the plate.

1. Pipet **100 ul** standards and diluted samples in duplicate into appropriate wells. Mix gently, and incubate on at orbital micro-plate shaker at 150 rpm at room temp.. (18-25 oC) for **45 minutes.**

   Note: for ease of loading samples it is recommended that a second uncoated microwell plate should be used keeping diluted samples. This enables standards or samples to be transferred quickly to the ELISA plate using multichannel pipette.

2. Wash the wells **5 times with 400 ul** of 1x wash buffer.

3. Pipette **100 ul** of HRP-conjugate into each well. Mix gently, and incubate on at orbital micro-plate shaker at 150 rpm for **45 minutes** at room temperature.

4. Aspirate and wash the wells **5 times** with 1x wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing. Remove the traces of wash buffer by adsorbing the plate over a clean paper towels.

5. Add **100 ul of TMB** Substrate into each well. Mix gently. Cover the plate and incubate on plate shaker at 150 rpm for **20 minutes** at room temperature. Blue color develops.

6. Stop the reaction by adding **100 ul** of stop solution to all wells. Mix gently. Blue color turns yellow.

7. Measure the absorbance at **450 nm** using an ELISA reader. Color is stable for at least 30 minutes after stopping.

NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 2-8^{o}C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each wells the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.