Related Items

Catalog#	Description
7520	Goat IgG ELISA Kit, 96 tests, Quantitative
7520-RDT-25	TruStrip RDT Goat IgG Rapid Test cards, 10/pk
7530	Goat IgM ELISA Kit, 96 tests, Quantitative
7540	Goat IgA ELISA Kit, 96 tests, Quantitative
7550	Goat IgE ELISA Kit, 96 tests, Quantitative
7610-Fab	Sheep/Ovine Fab ELISA Kit, 96 tests, Quantitative
7615-Fc	Sheep IgG-Fc ELISA Kit, 96 tests, Quantitative
7620	Sheep IgG ELISA Kit, 96 tests, Quantitative
7620-RDT-25	TruStrip RDT Sheep IgG Rapid Test cards, 10/pk
7630	Sheep IgM ELISA Kit, 96 tests, Quantitative
7640	Sheep IgA ELISA Kit, 96 tests, Quantitative
7650	Sheep IgE ELISA Kit, 96 tests, Quantitative
6430	Rat IgG1 ELISA Kit, 96 tests, Quantitative
6440	Rat IgG2a ELISA Kit, 96 tests, Quantitative
6450	Rat IgG2b ELISA Kit, 96 tests, Quantitative
6470	Rat IgE ELISA Kit, 96 tests, Quantitative
6480	Rat IgM ELISA Kit, 96 tests, Quantitative
6490	Rat Alpha-1 Glycoprotein (A1-AGP) ELISA kit 96 tests, Quantitative
600-660-RMY	Rat Myoglobin ELISA Kit
1800	Human IgE ELISA Kit, 96 tests, Quantitative
300-190-CGE	Cat IgE ELISA Kit, 96 tests, Quantitative
400-190-DGE	Dog IgE ELISA Kit, 96 tests, Quantitative
6470	Rat IgE ELISA Kit, 96 tests, Quantitative
7070	Monkey IgE ELISA Kit, 96 tests, Quantitative
7075	Chimp IgE ELISA Kit, 96 tests, Quantitative
7750	Horse IgE ELISA Kit, 96 tests, Quantitative

Monkey: IgM, IgG, IgA, IgE
Rat: Albumin, CRP, IgG, IgM, Alpha-1- Acid glycoprotein
Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgE, IgG3, IgE, IgM, Leptin, Resistin, Acrp30, CRP, Troponin-I, TNF-alpha

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 **Rabbit**: CRP, IgG
See Details at the web site or Contact ADI

Instruction Manual No. M-7530

Goat IgM ELISA KIT

Cat. # 7530, 96 Tests

For measurement of IgM in goat serum or plasma

For research use only (RUO), not for diagnosis, cure or prevention of the disease.

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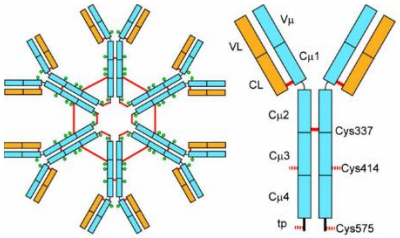
Goat IgM ELISA KIT # 7530

Kit Components, 96 tests	
Anti-goat IgM coated strip plate (8 wells x 12 strips), # 7531P, **Store at -20°C**	1 plate
Goat IgM Stock, Lyophilized, **Store at -20°C** , # 7532	3 vials
HRP Conjugate Conc., **50 µl**, # 7533, **Store at -20°C**	1 vial
Sample Diluent (10X), 25 ml, # 7534	1 bottle
Wash Buffer (20X), 50 ml, # WB-7530	1 bottle
TMB Substrate, 11 ml, # TMB-7530	1 bottle
Stop solution, 11 ml, # SS-7530	1 bottle
Instruction Manual, # M-7530	1

Intended Use

ADI's Goat IgM ELISA kit is a sandwich ELISA for measurement of IgM in goat serum or plasma**.** This kit is for **in vitro research use only (RUO).**

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.

Quality Control

Full set of reference standards must be run with each run. Reference standard should closely reflect the values shown in this manual. Blanks must be less than A450=0.300. Higher blanks is an indication of poor washing. Repeat the stds only with proper washing to confirm the expected values.

PERFORMANCE CHARACTERISTICS

Wash Procedure: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

Expected Values: Each laboratory should establish testing ranges for the animal population being investigated.

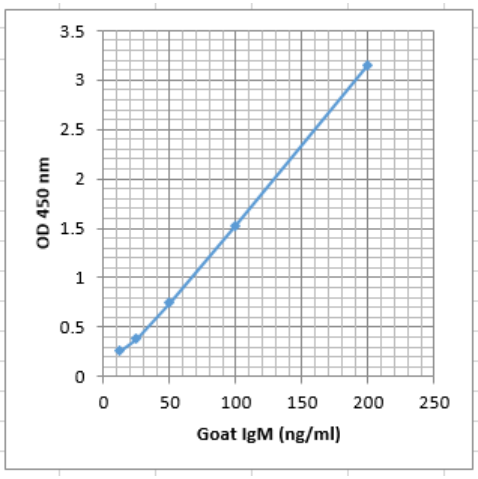
Species Cross-reactivity

This kits has not been tested with species other than goat. ADI has separate IgM kits for human, monkey, mouse, and other species.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples		Mean A_{450} nm	Calculated Concn
A1, A2	**Diluent**	0 ng/ml		
B1, B2	**Standard A**	12.5 ng/ml	0.257	
C1, C2	**Standard B**	25 ng/ml	0.380	
D1, D2	**Standard C**	50 ng/ml	0.746	
E1, E2	**Standard D**	100 ng/ml	1.563	
F1, F2	**Standard E**	200 ng/ml	3.152	

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.



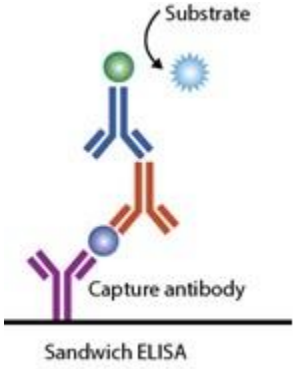
*7_ADI_ELISA-7530-ELISA

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

CALCULATION OF RESULTS:

1.Calculate the average absorbance values (A450) for blanks and each set of reference standards and samples.
2. Construct a standard curve by plotting the net mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of IgM in ng/ml from the standard curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of IgE in the sample.
5. Ideally, PC graphing software may be used for the above steps. We find good fits of standard curve data to a one site –total and nonspecific binding model.
6. If the OD450 values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

PRINCIPLE OF THE TEST



Goat IgM ELISA kit is based on binding of Goat 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. Plate shaker or orbital shaker; Reagent troughs, plate washer (recommended) and ELISA plates Reader.

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 TMB, sulfuric acid and BND can be requested or obtained from the ADI website: Sample Diluent and anti-Protein G-HRP contain Proclin 300 (0.05%, v/v). http://4adi.com/objects/catalog/product/extras/ELISA-Kit-SDS-MSDS-Set-1.pdf

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 cannot be immediately assayed, store frozen for up to six months. Avoid repeated freezing and thawing of samples. Cell or tissues extract samples have not been optimized.

Sample Preparation

IgM is typically present in goat 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 25,000 fold using the following procedure. This is done in 2 steps:

1. Prepare a dilution of 1:250 (Dispense 498 ul sample diluent into a tube and mix it with 2.00 ul of sample.

2. Prepare a dilution of 1:25000 from 1:250 sample: Mix 5.0 ul of the 250 fold diluted sample with the 495 ul of diluent in the second tube. This provides a 25,000 fold dilution of the sample.

Note: Some samples may have to be diluted more or less to bring them within the range of the curve. Users must dilute samples according to the amounts of goat IgM present in the sample. So final sample dilution may differ from the suggested 1:25,000.

REAGENT PREPARATION

1. **Dilute Wash Buffer (20x stock)**. Dilute the entire 50 ml with 950 ml of distilled or deionized water (total volume 1000 ml). Store at room temperature for the entire use of the kit. It can be stored at 4ºC for long term storage.

2. **Sample Diluent** is 10X. **Dilute 1:10** with water (1 ml stock in 9 ml water). Store 1x sample diluent at 4oC. Prepare as necessary and store the rest of the stock at 2-4ºC.

3. **HRP Conjugate:** The HRP conjugate should be prepared approximately five minutes before required. The HRP conjugate stock should be **diluted with diluent as detailed on the stock vial label.**

4. **Preparation of Standards:**

The goat IgM standard is provided as a **lyophilized stock**. **As per detail provided on vial** in distilled or deionized water (the reconstituted standard is stable at 4oC for one day but should be aliquoted and frozen at -20oC after reconstitution if future use is intended).

1. Label 5 polypropylene or glass tubes as 200, 100, 50, 25, and 12.5 ng/ml.

2. Into the tube labeled 200 ng/ml, pipette the volume of diluent detailed on the IgM standard vial label. Then add the indicated volume of IgM standard (shown on the IgM standard vial label) and mix gently. This provides the 200 ng/ml standard.

3. Dispense 250 ul of diluent into the tubes labeled 100, 50, 25 & 12.5 ng/ml.

4. Prepare a 100 ng/ml standard by diluting and mixing 250 ul of the 200 ng/ml standard with 250 ul of diluent in the tube labeled 100 ng/ml.

5. Similarly prepare the 50, 25 & 12.5 ng/ml standards by 2-fold serial dilution.

Notes: When preparing the serial dilutions of the standards gently mix the standards for 5-10 seconds and then take aliquots to make further dilutions. Following the above dilution scheme.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8ºC until the expiration date printed on the label. The Goat IgM reference standard should be stored at -20ºC.

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. Use first 2 wells for blanks (100 ul of 1x sample diluent). Pipet **100 ul standards and samples** in duplicate into appropriate wells. Mix gently for 5-10 seconds, and incubate at room temperature (25°C) for **45 minutes on an orbital shaker (150 rpm).** If an automated shaker is not available, the plate can be mixed manually every few minutes.

2. Remove or aspirate the plate contents and **wash the wells 5 times** with 400 ul of 1x wash buffer using an automated washer. If washing manually then dump the plate contents and tap over paper towels, add wash buffer, shake the contents of 5-10 seconds and repeat the steps. Tap the plate over fresh paper towels between each washing.

3. Pipette **100 ul of HRP conjugate** into each well, and incubate at room temperature (25°C) for **45 minutes on an orbital shaker (150 rpm).**

4. Remove or aspirate the plate contents and **wash the wells 5-6 times** with 400 ul of 1x wash buffer as above in step 5.

5. **Add 100 ul of TMB Substrate** into each well. Mix gently for 5-10 seconds. Cover the plate and incubate for **20 minutes** at 25°C **on an orbital shaker (100-150 rpm)**. **Blue color develops in standards and positive wells**. This step can be reduced or increased by ± 5 minutes to keep the color within reading range. If your ELISA reader cannot read above A450 of 2.00-3.00 then reduce the incubation time.

6. Stop the reaction by adding **100 ul of stop solution** to all wells. Mix gently for 5-10 seconds. **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.

Please Note: Due to plate reader differences, the high standard absorbance values may be out of range occasionally. If this occurs, absorbance values may be determined at 405 nm instead. If absorbance values exceed the high standard, the samples should be appropriately diluted and re-examined. Samples with absorbance values below those of the lowest standard should be assigned a zero IgA value.