ELISA kits available from ADI:

Catalog#	ProdDescription
6240	Mouse Serum Amyloid A ELISA Kit
6250	Mouse Serum Haptoglobin ELISA Kit
6250-10	Dog Serum Haptoglobin ELISA Kit
6250-20	Horse Serum Haptoglobin ELISA Kit
6250-30	Rat Serum Haptoglobin ELISA Kit
6250-50	Cat Serum Haptoglobin ELISA Kit
6250-60	Bovine Serum Haptoglobin ELISA Kit
600-480-CTN	Rabbit Cardiac Tn-I ELISA kit for serum samples
600-510-MTN	Rat Skeletal Muscle Troponin 1 (Tn-I) ELISA Kit
600-600-DMY	Dog Myoglobin ELISA Kit
600-610-HMY	Human Myoglobin ELISA Kit
600-620-MMY	Monkey Myoglobin ELISA Kit
600-630-MMY	Mouse Myoglobin ELISA Kit
600-640-PMY	Pig Myoglobin ELISA Kit
600-650-RMY	Rabbit Myoglobin ELISA Kit

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, Troponin-I, TNF-alpha

Autoimmune Antibody detection kits for ANA, ssDNA, dsDNA, Histone, Sm, RNP, SSA, SSB, Scl70, Ovalbumin, Cardiolipin, CIC

Chicken: IgG, IgM, IgY, Ovalbumin **Turkey**: IgG
Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin
Pig: Albumin, IgG, IgM **Dog:** CRP, IgG, IgM
Cat: IgG, IgM **Sheep:** IgG **Goat:** IgG **Rabbit**: CRP, IgG
See Details at the web site or Contact ADI

Instruction Manual No. M-7715-Fc

Horse IgG ELISA KIT

Cat. No. 7715-FC, 96 tests

For Quantitative Determination of horse IgG-Fc in Horse serum or plasma or other biological fluids

ALPHA DIAGNOSTIC INTERNATIONAL

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Your Molecular & Cell Technology Partner

Draft version-Please consult the manual supplied with the kit for any lot specific change.

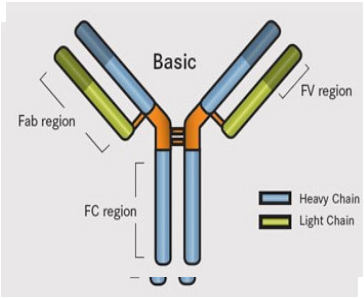
Horse IgG ELISA KIT Cat. No. 7715-Fc

Kit Components, 96 tests	
Anti-Horse IgG **coated strip** plate (8 wells x 12 strips), #7715	1 plate
Horse IgG **Calibrator**, **lyophilized**, *Reconstitute with **1 ml dH20** according to vial label, #7716*	1 vial
Anti-Horse IgG-**HRP Conjugate(100X)**, **150 ul,** #7717	1 bottle
Wash Buffer (20X), 50 ml, #7730-WB	1 bottle
Sample Diluent Concentrate, (5X) 50 ml, #7730-SD	1 bottle
TMB Substrate, 12 ml, #7730-TMB	1 bottle
Stop solution, 12 ml, #7730-SS	1 bottle
Instruction Manual, #M-7730	1 manual

Intended use:

ADI's horse IgG-Fc ELISA provides is a rapid, specific and sensitive assay for measuring horse IgG-Fc in serum, plasma or other biological solutions. Research Use Only (RUO), not for therapeutic use.

INTRODUCTION:



Immunoassays using heavy-chain specific antibodies provide for selective, sensitive quantification of human immunoglobulins IgG, IgA and IgM, as found circulating in blood or as present in other body fluids, including saliva, milk/colostrum, ascites, tears and mucosa of linings of the gut, respiratory or urigenital tracts.

Levels of total IgG, IgA and/or IgM can reveal health status or results of experimental or pathological conditions (e.g., hypo- or hypergammaglobulinemia or acute or chronic infection). Also, measurements of specific antibody levels, in antigen-specific assays, are often best interpreted relative to values of total IgG, IgA, and IgG in the sample and/or individual.

The quantitative immunoassays measure human IgG, IgA and IgM with high sensitivity; this allows for sufficient dilution of the sample to avoid sample matrix interference that may occur with any of the above specimen types. Also, each assay is Ig class specific, such that all IgG or IgA subclasses are reliably quantified in essentially any specimen, freshly obtained and/or suitable stored. Expected performance of each kit relative to precision, recovery and linearity of dilution is presented as guidance for use and experimental design

CALCULATION OF RESULTS

Subtract the average background value from the test values for each sample. Using the results observed for the standards construct a Standard Curve. The appropriate curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fits may also be used.

Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the horse IgG concentration in original samples.

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.

Specificity

The antibodies used in this kit are specific for horse IgG-Fc. The kit will measure both whole IgG or IgG-Fc.

Species Crossreactivity:

Cross-reactivity with other species is not tested. ADI has IgG ELISA kits for mouse, rat, dog, horse, pig, cat, bovine, and monkey.

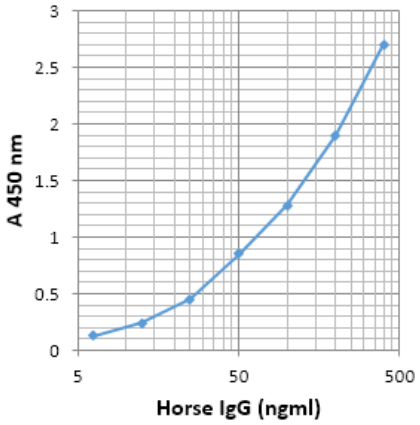
NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and 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 well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples		Mean $A_{450\ nm}$	Calculated Concn
A1, A2	**Standard A**	0 ng/ml	0.05	
B1, B2	**Standard B**	6.2 ng/ml	0.13	
C1, C2	**Standard C**	12.5 ng/ml	0.24	
D1, D2	**Standard D**	25.5 ng/ml	0.45	
E1, E2	**Standard E**	50 ng/ml	0.85	
F1, F2	**Standard F**	100 ng/ml	1.28	
G1, G2	**Standard G**	200 ng/ml	1.9	
H1, H2	**Standard H**	400 ng/ml	2.7	

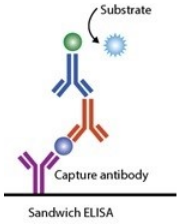
NOTE: These data are for demonstration purpose only. Actual values may vary slightly from above. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



7_Ar_Elisa

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

PRINCIPLE OF THE TEST



Horse IgG-Fc ELISA kit, a sandwich ELISA, is based on binding of IgG 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 IgG 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 IgG 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

The Human HAPTOGLOBIN ELISA Kit is for research use only.
Stop Solution contains 1% 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.

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. **Sample diluent concentrate (5X)** Dilute 1:5 (1 part diluent conc. With 4 parts distilled water). Dilute only the required reagent. Store diluted solution at 2-8^{o} C.

2. **Wash Buffer (20X stock)**. Dilute the entire **50 ml with distilled or deionized water to 950 ml water** (total volume 1000 ml). Store at room temperature for the entire use of the kit.

3. ENZYME-ANTIBODY CONJUGATE (100X)

Prepare 1X conjugate by diluting 100x stock (10 ul stock in 1 ml of sample diluent). Prepare 1 ml for each strip or 10 ml for entire plate. Prepare 1x stock as necessary and do not store 1X stock after the assay.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{o}C until the expiration date printed on the label. After opening the kit components, the shelf life is approximately 2 months.

DILUTION OF SAMPLES

The assay for quantification of horse IgG requires that each test sample be diluted before use. For a single step determination a dilution of serum/plasma at 1/200,000 is appropriate for most samples. For absolute quantification, samples that yield results outside the range of the standard curve, a lesser or greater dilution might be required. **If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.**

1. To prepare a 1/200,000 dilution of sample, transfer **2 µL** of sample to **1998 µL** of 1X diluent (dilution=1000) and mix gently. Next, dilute the 1/1000 samples by transferring **2 µL, to 398 µL** of 1X diluent (final dilution 1/200,000). Mix thoroughly at each stage.

Repeat this procedure for each sample to be tested

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

1. Reconstitute the lyophilized calibrator with **1 ml of distilled** or deionized water indicated on the vial. This gives the concentration **of the reference standard as 4.94 mg/ml (lot specific concn will be specified on the vial). Immediately aliquot and store** any unused reference standard at –20oC or below.

2. Prepare liquid standards using the following dilution scheme. Label 8 microcentrifuge tubes as 400, 200, 100, 50, 25, 12.5, 6.25, and 0 ng/ml.

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 dilution scheme.

Standard	ng/ml	Volume added to 1x Diluent	Volume of 1X Diluent
A	49400	5 µl Horse IgG calibrator	495 µl
7	400	6 µl Std A	735 µl
6	200	300 µl standard 7	300 µl
5	100	300 µl standard 6	300 µl
4	50.00	300 µl standard 5	300 µl
3	25.00	300 µl standard 4	300 µl
2	12.5	300 µl standard 3	300 µl
1	6.25	300 µl standard 2	300 µl
0	0		600 µl

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

3. Pipet **100 uL standards and diluted samples** into appropriate wells. Mix gently, and incubate at **room temperature** (20-25oC) for **30 minutes**.

4. Remove or aspirate the plate contents and **wash the wells 3 times** with 300 uL of distilled or deionized water using an automated washer. If washing manually then dump the plate contents and tap over paper towels, add water, shake the contents of 5-10 seconds and repeat the steps. Tap the plate over fresh paper towels between each washing.

5. Pipet **100 uL of 1x enzyme conjugate** into each well. Mix gently, and incubate for **20 minutes** at room temperature as in step 3.

6 **Wash the wells 3 times** as in step 4. Tap the plate over fresh paper towels to remove traces of liquid from the last washing step.

7. **Add 100 uL of TMB Substrate** into each well. Mix gently. Cover the plate and incubate in the dark for **10 minutes** at room temperature. Blue color develops. 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 then reduce the incubation time.

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

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