Related Items

Catalog#	Description
7710-Fab	Horse Fab2 ELISA Kit, 96 tests, Quantitative
7715-Fc	Horse IgG-Fc ELISA Kit, 96 tests, Quantitative
7720	Horse IgA ELISA Kit, 96 tests, Quantitative
7730	Horse IgG ELISA Kit, 96 tests, Quantitative
7730-RDT-10	TruStrip RDT Horse/Foal IgG (Failure of passive transfer, FPT) Rapid test cards, 10/pk
7740	Horse IgM ELISA Kit, 96 tests, Quantitative
7740-RDT-10	TruStrip RDT Horse IgM (immunodeficiency syndrome) Rapid test cards, 10/pk
7750	Horse IgE ELISA Kit, 96 tests, Quantitative

For more details please consult our web site (www.4adi.com) or contact us by email (service@4adi.com).

Horse Fab2 ELISA Kit

Cat. No. 7710-Fab, 96 Tests

For Quantitative Determination of Horse IgG-Fab2 in purified Fab2 preparations.

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

ALPHA DIAGNOSTIC INTERNATIONAL

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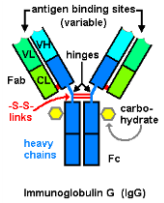
INTENDED USE

The Horse Fab2 ELISA Kit is a sandwich ELISA for the quantification of IgG-Fab2 in purified or semi-purified Fab2 preparation in appropriately qualified samples such horse Fab2 for anti-tetanus or diphtheria or other anti-toxins made in hose sera. For research use only (RUO), not for diagnosis, cure or prevention of the disease.

RESEARCH USE OF THE TEST

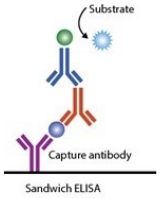
Passive immunity is the transfer or infusion of pre-made antibodies. Passive immunity also occur naturally, when maternal antibodies are transferred to the fetus through the placenta. Typically, high levels of antibodies specific to a pathogen (rabies), (diphtheria) or venom are made in animals that can provide large volumes (Horse or sheep or goat). Antibodies can be used infused an unpurified antiserum, purified IgG or IgG-Fab2 fragment to animals) are transferred to non-immune persons through infusion of immunoglobulin called antiserum therapy or immunoglobulin for intravenous (IVIG). Passive immunization is used when there is a high risk of infection and insufficient time for the body to develop its own immune response, or to reduce the symptoms of ongoing or immunosuppressive diseases. Passive immunization can be provided when people cannot synthesize antibodies, and when they have been exposed to a disease that they do not have immunity against.

IVIG may be obtained from human (CMV, Hepatitis A, B, rabies, tetanus, vaccinia or varicella) or made in large donor species such as horse (Botulism, diphtheria, rabies, tetanus, and anti-venom). Infusion of large volumes of IVIG from animals to humans may invoke prophylactic shock. Therefore, many IVIG use partially purified whole IgG or fragments of IgG-Fab2 (e.g. equine rabies anti-tetanus, equine anti-diphtheria, and FAV Africa, equine F(ab')2 against the snake venom). ADI has developed antibody ELISA kits to measure antibody titers in various IVIG and also develop an ELISA kit to determine the concentration of equine Fab2. Equine IgG-Fab2 ELISA will work in purified antibodies or when they contain other protein additives.



Immunoassays using heavy-chain specific antibodies provide for selective, sensitive quantification of Horse 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 urogenital tracts. The quantitative immunoassays measure Horse Fab2 and Horse IgG with high sensitivity; this allows dilution beyond interference from the sample matrix for samples derived from any of the above specimen types. Expected performance of each kit relative to precision, recovery and linearity of dilution is presented as guidance for use and experimental design.

PRINCIPLE OF THE TEST



The Horse Fab2 ELISA kit is based on the binding of Horse Fab2 in samples to two antibodies, one immobilized on the microtiter wells, and the other conjugated to horseradish peroxidase (HRP) enzyme. After a washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of IgG present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of IgG in samples and control is calculated from a curve of standards containing known concentrations of IgG.

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 box label. Stabilities of the working solutions are indicated under Reagent Preparation.

PERFORMANCE CHARACTERISTICS

Normal Range

Assay of IgG in pooled sera ranged from ~9 mg/ml. Each laboratory should determine expected values of its own testing population.

Tetanus Anti-Toxin (ATS)

A commercial preparation of horse anti-tetanus toxin, Fab2 was tested in the kit (VINS #1000 IU). It registered a concentration of ~20 mg/ml. We recommend that users test their own Horse-Fab2 preparations as an internal control.

Specificity

The affinity purified antibody used in this kit resulted in a single precipitin arc against anti-Peroxidase, anti-Goat Serum, Horse IgG (whole), Horse IgG F(ab')2 or IgG-Fab' and Horse Serum. No reaction was observed against Horse IgG F(c), and have essentially no reactivity with IgM, IgA, IgE or any other Horse serum proteins.

Species Crossreactivity

Normal pooled serum samples were tested at 1:1000 dilution showed minimal or no cross reactivity in the Horse IgG-Fab2 ELISA.

Sensitivity

Based upon the values of the zero standard or blanks, the sensitivity of the kit is ~ 1.5 ng/ml for horse IgG-Fab2.

CALCULATION OF RESULTS

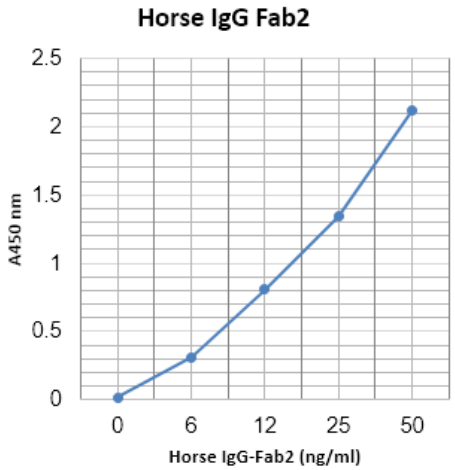
1. The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, IgG concentrations may be determined as follows:
2. Calculate the mean OD of duplicate samples.
3. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of IgG (x-axis). Draw the curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
4. The IgG concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
5. Multiply the values obtained for the samples by the dilution factor of each sample.
6. Samples producing signals higher than the 50 ng/ml standard should be further diluted and re-assayed.

TYPICAL RESULTS

The following data are for illustration purposes only. A complete standard curve should be run in every assay to determine sample values.

Wells	Standards, Control & Samples	Average A450 nm	Mean A450 nm
A1, A2	**Negative Diluent Control**	0.015	0
B1, B2	6 ng/ml **Standard**	0.31	0.29
C1, C2	12 ng/ml **Standard**	0.81	0.79
D1, D2	25 ng/ml **Standard**	1.34	1.32
E1, E2	50 ng/ml **Standard**	2.11	2.09
F1, F2	**Sample** [Diluted 1:50k]	1.5	1.48

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



3soumi/7710-Fab-ELISA-Graph

KIT CONTENTS

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190 ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8°C until the kit lot expires or is used up.
Wash Solution Concentrate (50x) Cat. No. WB-50, 10ml	Dilute the entire volume 10ml + 450 ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at RT until kit is used entirely.
Anti-Horse Fab2 - HRP Conjugate Concentrate (100x) Part No. 7712, 0.11ml	Peroxidase conjugated anti-Horse Fab2 in buffer with protein, detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent (WSD)** is sufficient for one 8-well strip. Use within the working day and discard. Return concentrate to 2-8°C storage.

Ready For Use: Store as indicated on labels.

Component	Part No.	Amt	Contents
Anti-Horse Fab2 Microwell Strip Plate	7711	8-well strips (12)	Coated with purified anti-Horse Fab2 antibodies.
Horse Fab2 Standards			
6 ng/ml 12 ng/ml 25 ng/ml 50 ng/ml	7713A 7713B 7713C 7713D	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Five (5) vials, each containing calibrated Horse Fab2 in buffer with protein, detergents and antimicrobial as stabilizers.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Diluted sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Horse Fab2-HRP Concentrate.
- Graduated cylinder to dilute Wash ConcN. and Sample Diluent concentrate; 200ml to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera are not assayed immediately , store refrigerated for up to 2 weeks, or frozen for long-term storage. Avoid freeze-thaw cycles.
The use of plasma has not been investigated, but should be a suitable specimen for assay.

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: Sample Diluent and anti-Protein G-HRP contain Proclin 300 (0.05%, v/v).

DILUTE Serum Samples in Working Sample Diluent. Dilutions of about 1:200,000-1:500,000 are appropriate for most normal Horse sera. For accuracy, three dilution steps are recommended, as follows:

1) 10 ul serum + 990 ul diluent = [1:100],
2) 10 ul [1:100] + 990 ul diluent = [1:1000],
3) 10 ul [1:1000] + 990 ul diluent = **1:100,000**
4) 50 ul of 1:100,000 + 200 ul of diluent =1:500,000.

Dilutions #1-3 can be done in normal saline or PBS or sample diluent but the test dilutions should be made in sample dilution to avoid any matrix effect.

Horse IgG-Fab2 Sample Dilution

If using purified horse Fab2 preparation, then dilute samples as per the initial stock concentration so as to bring the samples within the range of the kit.

QUALITY CONTROL

Reagents Accurate and reproducible assay results rely on proper storage, handling and control of reagent and sample temperature. Store all reagents as indicated, and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

Sample Controls Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Negative Diluent Control should also be run; OD < 0.3.

Standard Curve The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-continuously increasing or low signals may indicate problems with technique, protocol directions and/or reagent preparation.

ASSAY PROCEDURE

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

DO NOT diluted the Standards or Control.

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE (25-28Oc). After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. Set-up

Determine the number of wells for the assay run. Duplicates are recommended, including 10 Standard wells and 2 wells for each sample and 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 5 to 30 minutes before sample addition.
- Aspirate the liquid and pat dry on a paper towel.

2. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of standards, 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 is recommended. Improper washes may lead to falsely elevated signals and poor reproducibility.

3. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-Horse Fab2-HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

4. 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, assuring the top standard does not surpass 2 OD.

5. 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.

6. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm. 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.