



DRG[®] IFN- γ (Swine) ELISA (EIA-3178 / EIA-3179)

REVISED 4 JAN. 2007

FOR VETERINARY USE ONLY

1 INTENDED USE

The DRG[®] Swine Interferon- γ (Sw IFN- γ) ELISA is to be used for the in vitro quantitative determination of Sw IFN- γ in swine serum, plasma, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant Sw IFN- γ .

This kit has been configured for research use only and is not to be used in diagnostic procedures.

Read entire protocol before use.

2 PRINCIPLE OF THE METHOD

The DRG[®] Sw IFN- γ kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for Sw IFN- γ has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Sw IFN- γ content, control specimens, and unknowns, are pipetted into these wells, followed by the addition of a biotinylated monoclonal second antibody.

During the first incubation, the Sw IFN- γ antigen binds simultaneously to the immobilized (capture) antibody on one site, and to the solution phase biotinylated antibody on a second site.

After removal of excess second antibody, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a second incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of Sw IFN- γ present in the original specimen.

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3 REAGENTS PROVIDED

Note: Store all reagents at 2 - 8°C.

Reagent	96 Test Kit	192 Test Kit
Sw IFN- γ Standard , recombinant Sw IFN- γ . Refer to vial label for quantity and reconstitution volume.	2 vials	4 vials
Standard Diluent Buffer . Contains 15 mM sodium azide; 25 mL per bottle.	1 bottle	2 bottle
Sw IFN- γ Antibody-Coated Wells , 96 wells per plate.	1 plate	2 plate
Sw IFN- γ Biotin Conjugate , (Biotin-labeled anti-IFN- γ). Contains 15 mM sodium azide; 6 mL per bottle.	1 bottle	2 bottle
Streptavidin-Peroxidase (HRP) , (100x) concentrate. Contains 3.3 mM thymol; 0.125 mL per vial.	1 vial	2 vial
Streptavidin-Peroxidase (HRP) Diluent . Contains 3.3 mM thymol; 25 mL per bottle.	1 bottle	1 bottle
Wash Buffer Concentrate (25x) ; 100 mL per bottle.	1 bottle	1 bottle
Stabilized Chromogen , Tetramethylbenzidine (TMB); 25 mL per bottle.	1 bottle	1 bottle
Stop Solution ; 25 mL per bottle.	1 bottle	1 bottle
Plate Covers , adhesive strips.	3	4

Disposal Note: This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

4 SUPPLIES - NOT PROVIDED

1. Microtiter plate reader capable of measurement at or near 450 nm.
2. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
3. Distilled or deionized water.
4. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
5. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log, or semi-log, as desired.
6. Glass or plastic tubes for diluting and aliquoting standard.
7. Absorbent paper towels.
8. Calibrated beakers and graduated cylinders in various sizes.

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9. 37°C incubator.

5 PROCEDURAL NOTES/LAB QUALITY CONTROL

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 - 8°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin-free tubes.
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all standards, controls and samples be run in duplicate.
7. Samples that are >500 pg/mL should be diluted with Standard Diluent Buffer.
8. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
9. Cover or cap all reagents when not in use.
10. **Do not mix or interchange different reagent lots from various kit lots.**
11. Do not use reagents after the kit expiration date.
12. Read absorbances within 2 hours of assay completion.
13. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
14. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
15. Because Stabilized Chromogen is light sensitive, avoid prolonged exposure to light. Also avoid contact between Stabilized Chromogen and metal, or color may develop.

6 SAFETY

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

7 DIRECTIONS FOR WASHING

Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Buffer provided.

Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.

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After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under ASSAY METHOD. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

If using an automated washer, the operating instructions for washing equipment should be carefully followed.

8 REAGENT PREPARATION AND STORAGE

8.1 Reconstitution and Dilution of Sw IFN- γ Standard

Note: Either glass or plastic tubes may be used for standard dilutions.

1. Reconstitute standard to 2500 pg/mL with Standard Diluent Buffer. Refer to standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Use standard within 1 hour of reconstitution.
2. Add 0.150 mL of the reconstituted standard to a tube containing 0.600 mL Standard Diluent Buffer. Label as 500 pg/mL. Mix.
3. Add 0.300 mL of Standard Diluent Buffer to each of 6 tubes labeled 250, 125, 62.5, 31.2, 15.6 and 7.8 pg/mL Sw IFN- γ .
4. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

8.1.1 Dilution of Sw IFN- γ Standard

Standard:	Add:	Into:
500 pg/mL	Prepare as described in Step 2.	
250 pg/mL	0.300 mL of the 500 pg/mL std.	0.300 mL of the Diluent Buffer
125 pg/mL	0.300 mL of the 250 pg/mL std.	0.300 mL of the Diluent Buffer
62.5 pg/mL	0.300 mL of the 125 pg/mL std.	0.300 mL of the Diluent Buffer
31.2 pg/mL	0.300 mL of the 62.5 pg/mL std.	0.300 mL of the Diluent Buffer
15.6 pg/mL	0.300 mL of the 31.2 pg/mL std.	0.300 mL of the Diluent Buffer
7.8 pg/mL	0.300 mL of the 15.6 pg/mL std.	0.300 mL of the Diluent Buffer
0 pg/mL	0.300 mL of the Diluent Buffer	An empty tube

Discard all remaining reconstituted and diluted standards after completing assay. Return the Standard Diluent Buffer to the refrigerator.

8.2 Storage and Final Dilution of Streptavidin-HRP

Please Note: The Streptavidin-HRP 100x concentrate is in 50% glycerol. This solution is viscous. To ensure accurate dilution, allow Streptavidin-HRP concentrate to reach room temperature. Gently mix. Pipette Streptavidin-HRP concentrate slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

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1. Dilute 10 μ L of this 100x concentrated solution with 1 mL of Streptavidin-HRP Diluent for each 8-well strip used in the assay. Label as Streptavidin-HRP Working Solution.

For Example:

# of 8-Well Strips	Volume of Streptavidin-HRP Concentrate	Volume of Diluent
2	20 μ L solution	2 mL
4	40 μ L solution	4 mL
6	60 μ L solution	6 mL
8	80 μ L solution	8 mL
10	100 μ L solution	10 mL
12	120 μ L solution	12 mL

2. Return the unused Streptavidin-HRP concentrate to the refrigerator.

8.3 Dilution of Wash Buffer

Allow the 25x concentrate to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the 25x wash buffer concentrate with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.

Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

9 ASSAY METHOD: PROCEDURE AND CALCULATIONS

Be sure to read the Procedural Notes/Lab Quality Control section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Add 100 μ L of the Standard Diluent Buffer to zero wells. Well(s) reserved for chromogen blank should be left empty.
3. For the standard curve, add 100 μ L of standards to the appropriate microtiter wells. For all samples (controls, serum, plasma, buffered solution and cell culture medium), add 50 μ L of Standard Diluent Buffer to each well followed by 50 μ L of sample. (See REAGENT PREPARATION AND STORAGE.)
4. Pipette 50 μ L of biotinylated anti-IFN- γ (Biotin Conjugate) solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.
5. Cover plate with plate cover and incubate for **2 hours at 37°C**.
6. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See DIRECTIONS FOR WASHING.

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7. Add 100 μ L Streptavidin-HRP Working Solution to each well except the chromogen blank(s). (Prepare the working dilution as described in REAGENT PREPARATION AND STORAGE.)
8. Cover plate with the plate cover and incubate for **30 minutes at room temperature**.
9. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See DIRECTIONS FOR WASHING.
10. Add 100 μ L of Stabilized Chromogen to each well. The liquid in the wells will begin to turn blue.
11. Incubate for **30 minutes at room temperature and in the dark**.
Please Note: Do not cover the plate with aluminum foil or metalized mylar. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the Stop Solution has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.
12. Add 100 μ L of Stop Solution to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
13. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 μ L of Stabilized Chromogen and 100 μ L of Stop Solution. Read the plate within 2 hours after adding the Stop Solution.
14. Plot on graph paper the absorbance of the standards against the standard concentration. (Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.) Draw the best smooth curve through these points to construct the standard curve. If using curve fitting software, the four parameter algorithm provides the best curve fit.
15. Read the Sw IFN- γ concentrations for unknown samples and controls from the standard curve plotted in step 14. **Multiply value(s) obtained for samples by 2 to correct for the 1:2 dilution in step 3.** (Samples producing signals greater than that of the highest standard (500 pg/mL) should be further diluted in Standard Diluent Buffer and reanalyzed, multiplying the concentration found by the appropriate dilution factor.)

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TYPICAL DATA

The following data were obtained for the various standards over the range of 0 to 500 pg/mL Sw IFN- γ .

Standard Sw IFN- γ (pg/mL)	Optical Density (450 nm)
0	0.084 0.083
7.8	0.123 0.127
15.6	0.153 0.157
31.2	0.239 0.247
62.5	0.433 0.421
125	0.784 0.770
250	1.414 1.356
500	2.447 2.597

10 LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 500 pg/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples >500 pg/mL with Standard Diluent Buffer; reanalyze these and multiply results by the appropriate dilution factor.

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated. The rate of degradation of native Sw IFN- γ in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

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11 PERFORMANCE CHARACTERISTICS

11.1 SENSITIVITY

The minimum detectable dose of Sw IFN- γ is <2.0 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

11.2 PRECISION

1. Intra-Assay Precision

Samples of known Sw IFN- γ concentration were assayed in replicates of 14 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	81.7	158.0	334.9
SD	2.6	7.8	15.4
%CV	3.2	4.9	4.6

SD = Standard Deviation

CV = Coefficient of Variation

2. Inter-Assay Precision

Samples were assayed 42 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	83.0	153.1	326.7
SD	7.3	11.0	24.3
%CV	8.8	7.2	7.4

11.3 LINEARITY OF DILUTION

Swine serum and tissue culture medium containing 10% fetal bovine serum were spiked with Sw IFN- γ and serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 in both cases.

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Dilution	Serum		Cell Culture	
	Measured (pg/mL)	Expected (pg/mL)	Measured (pg/mL)	Expected (pg/mL)
1/2	473	-	353	-
1/4	193	236	189	177
1/8	92	118	94	88
1/16	53	59	50	44
1/32	25	30	24	22
1/64	12	15	11	11

11.4 RECOVERY

The recovery of Sw IFN- γ added to swine serum averaged 80%. The recovery of Sw IFN- γ added to plasma averaged 75%. The recovery of Sw IFN- γ added to tissue culture medium containing 1% fetal bovine serum averaged 119%, while the recovery of Sw IFN- γ added to tissue culture medium containing 10% fetal bovine serum averaged 109%.

Sera and plasma from Yorkshire and Chester-White pigs have been validated for use in this assay. Other strains of swine have not been tested and consequently their use has not been validated.

11.5 SPECIFICITY

Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the DRG[®] Sw IFN- γ kit.

The following substances were tested and found to have no cross-reactivity:

human IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IFN- γ ;

mouse IL-1 β , IL-2, IL-3, IL-4, IFN- γ ;

rat IFN- γ , TNF- α ;

swine IL-1 β , IL-8, IL-10.

11.6 HIGH DOSE HOOK EFFECT

A sample spiked with Sw IFN- γ up to 7 ng/mL gives a response higher than that obtained for the last standard point.

11.7 EXPECTED VALUES

Swine PBMCs were cultured under the following conditions and the culture supernatants were assayed for Sw IFN- γ released.

1. Unstimulated: 0 pg/mL
2. Con-A (5 μ g/mL) 22 hr: 290 pg/mL
3. PHA (10 μ g/mL) 32 hr: 1400 pg/mL
4. PHA (10 μ g/mL) 48 hr: 1600 pg/mL
5. PHA (10 μ g/mL) 72 hr: 2400 pg/mL
6. PMA (50 ng/mL), Ionophore (250 ng/mL) 5 hr: 1100 pg/mL

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7. PMA (50 ng/mL), Ionophore (250 ng/mL) 15 hr: 7400 pg/mL
8. PMA (50 ng/mL), Ionophore (250 ng/mL) 15 hr, then, LPS (10 µg/mL) 4 hr: 8100 pg/mL

SYMBOLS USED WITH DRG ELISA 'S

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
	European Conformity	CE-Konformitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	Research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Distributeur	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità
Symbol	Portugues	Dansk	Svenska	Ελληνικά	
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη	
	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση	
	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό	
					
	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου	
	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος	
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις	
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstemperatur	Θερμοκρασία αποθήκευσης	
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης	
	Fabricante	Producent	Tillverkare	Κατασκευαστής	
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο	