



## **ELISA PRODUCT INFORMATION & MANUAL**

### **HLA G *NBP2-62174***

Enzyme-linked Immunosorbent Assay for quantitative  
detection of Human HLA G.

For research use only.

Not for diagnostic or therapeutic procedures.

[www.novusbio.com](http://www.novusbio.com) - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - [technical@novusbio.com](mailto:technical@novusbio.com)

Novus kits are guaranteed for 6 months from date of receipt

# Product Manual

## **USE FOR RESEARCH PURPOSES ONLY**

Unless otherwise specified expressly on the packaging, all products sold hereunder are intended for and may be used for research purposes only and may not be used for food, drug, cosmetic or household use or for the diagnosis or treatment of human beings. Purchase does not include any right or license to use, develop or otherwise exploit these products commercially. Any commercial use, development or exploitation of these products or development using these products without the express written authorization of Novus Biologicals is strictly prohibited. Buyer assumes all risk and liability for the use and/or results obtained by the use of the products covered by this invoice whether used singularly or in combination with other products.

## **LIMITED WARRANTY; DISCLAIMER OF WARRANTIES**

These products are offered under a limited warranty. The products are guaranteed to meet all appropriate specifications described in the package insert at the time of shipment. Novus Biologicals' sole obligation is to replace the product to the extent of the purchasing price. All claims must be made to Novus Biologicals, within five (5) days of receipt of order. THIS WARRANTY IS EXPRESSLY IN LIEU OF ANY OTHER WARRANTIES OR LIABILITIES, EXPRESS OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, AND NON-INFRINGEMENT OF THE PATENT OR OTHER INTELLECTUAL PROPERTY RIGHTS OF OTHERS, AND ALL SUCH WARRANTIES (AND ANY OTHER WARRANTIES IMPLIED BY LAW) ARE EXPRESSLY DISCLAIMED.

## **TRADEMARKS AND PATENTS**

Several Novus Biologicals products and product applications are covered by US and foreign patents and patents pending. Novus Biologicals is a trademark of Novus Biologicals

**FOR RESEARCH USE ONLY.  
NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

# Product Manual



Please read entire booklet before proceeding with the assay.



Carefully note the handling and storage conditions of each kit component.



Please contact Novus Biologicals Technical Support if necessary.

## TABLE OF CONTENTS

Intended Use.....	2
Storage, Expiration.....	2
Introduction .....	3
Test Principle .....	4
Precautions .....	4
Technical Hints .....	5
Reagents Supplied.....	5
Materials Needed But Not Supplied .....	6
Preparation of Reagents .....	7
Preparation of Samples.....	9
Assay Procedure.....	10
Calculations .....	12
Typical Standard Curve.....	12
Performance Characteristics .....	13
Method Comparison.....	15
Troubleshooting & FAQs .....	15
References.....	16
Assay Procedure Summary.....	20
Plate Layout .....	21

# Product Manual

## INTENDED USE

The soluble HLA-G ELISA is a sandwich enzyme immunoassay for the quantitative measurement of soluble forms of human leukocyte antigen-G (sHLA-G).

## Features

- It is intended for research use only
- The total assay time is about 20 hours
- The kit measures shedded HLA-G1 and HLA-G5 in serum, plasma, amniotic fluid or cell culture supernatant
- Calibrator is human native protein
- Assay format is 96 wells
- Components of the kit are provided ready to use, concentrated or lyophilized

## STORAGE, EXPIRATION

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

For stability of opened reagents see **page 6**.

## INTRODUCTION

Human leukocyte antigen-G (HLA-G) differs from the other MHC class I genes by its low polymorphism and alternative splicing that generates seven HLA-G proteins, whose tissue-distribution is restricted to normal fetal and adult tissues that display a tolerogeneic function toward both innate and acquired immune cells. Soluble HLA-G is an immunosuppressive molecule inducing apoptosis of activated CD8(+) T cells and down-modulating CD4(+) T cell proliferation.

Recently, using specific ELISA to analyze the presence of soluble HLA-G molecules in culture supernatants of early embryos obtained by in vitro fertilization (IVF) before transfer, several reports demonstrated that positive embryo implantations occurred with embryos secreting soluble HLA-G molecules. These breakthrough results indicate that soluble HLA-G ELISA can be a useful biochemical assay in addition to embryo morphology in embryo selection for transfer in IVF treatment if there are other embryos with the same morphology.

Furthermore, monitoring of soluble HLA-G in amniotic fluid and plasma of pregnant women may have an important prognostic value to recognize pathological situations. Other interesting observations suggest that HLA-G molecules seem to be directly involved in transplant acceptance, and their analysis should be taken into consideration when monitoring transplant-patients status. In addition, soluble HLA-G plasma levels are increased in lymphoproliferative disorders or in patients suffering from malignant melanoma, glioma, breast and ovarian cancer.

### **Areas of investigation:**

Immune Response, Infection and Inflammation

Reproduction

Transplantation

# Product Manual

## TEST PRINCIPLE

In the Novus Biologicals soluble HLA-G ELISA, calibrators and samples are incubated in microplate wells pre-coated with monoclonal anti-soluble HLA-G antibody. After 16-20 hours incubation and washing, monoclonal anti-human  $\beta$ 2-microglobulin antibody labeled with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with captured soluble HLA-G. Following another washing step, the remaining HRP conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of soluble HLA-G. A calibration curve is constructed by plotting absorbance values against concentrations of calibrators, and concentrations of unknown samples are determined using this calibration curve.



Handle  
with care

## PRECAUTIONS

- For professional use only
- Wear gloves and laboratory coats when handling immunodiagnostic materials
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- This kit contains components of animal origin. These materials should be handled as potentially infectious
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary
- The materials must not be pipetted by mouth

# Product Manual

## TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware
- Use deionized (distilled) water, stored in clean containers
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light
- Stop Solution should remain colorless until added to the plate. The color developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

## REAGENT SUPPLIED

Kit Components	State	Quantity
Antibody Coated Microtiter Strips	ready to use	96 wells
Conjugate Solution Conc. (100X)	concentrated	0.13ml
Master Calibrator	lyophilized	2 vials
Conjugate Diluent	ready to use	13ml
Dilution Buffer 1	ready to use	20ml
Dilution Buffer 2	ready to use	13ml
Wash Solution Conc. (10X)	concentrated	100ml
Substrate Solution	ready to use	13ml
Stop Solution	ready to use	13ml
Product Data Sheet + Certificate of Analysis		1pc

# Product Manual

## MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution
- Precision pipettes to deliver 10-1000  $\mu$ l with disposable tips
- Multichannel pipette to deliver 100  $\mu$ l with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtiter plate after washing
- Vortex mixer
- Orbital microplate shaker capable of approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with  $450 \pm 10$  nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650nm)
- Software package facilitating data generation and analysis (optional)



# Product Manual

## PREPARATION OF REAGENTS

- All reagents need to be brought to room temperature prior to use
- Always prepare only the appropriate quantity of reagents for your test
- Do not use components after the expiration date marked on their label
- Assay reagents supplied ready to use:

### Antibody Coated Microtiter Strips

Stability and storage: Return the unused strips to the provided aluminium zip-sealed bag with desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months when stored at 2-8°C and protected from the moisture.

### Conjugate Solution

#### Dilution Buffer 1

#### Dilution Buffer 2

#### Substrate Solution

#### Stop Solution

Stability and storage: Opened reagents are stable 3 months when stored at 2-8°C.

- Assay reagents supplied lyophilized or concentrated:

### soluble HLA-G Master Calibrator

**Refer to the vial label for current volume of distilled water needed for reconstitution of calibrator!!!**

Reconstitute the lyophilized Master Calibrator with distilled water just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of the soluble HLA-G in the stock solution is **625 Units/ml**.

Prepare set of calibrators using Dilution Buffer 1 as follows (example for duplicates):

Volume of Calibrator	Dilution Buffer	Concentration
Stock	-	625.00 Units/ml
100µl of stock	400µl	125.00 Units/ml
250µl of 125.00 Units/ml	250µl	62.50 Units/ml
250µl of 62.50 Units/ml	250µl	31.25 Units/ml
250µl of 31.25 Units/ml	250µl	15.63 Units/ml
250µl of 15.63 Units/ml	250µl	7.81 Units/ml
250µl of 7.81 Units/ml	250µl	3.91 Units/ml

# Product Manual

**Prepared Calibrators are ready to use, do not dilute them.**

Stability and storage: Do not store the Calibrator stock solution and set of calibrators.

## **Conjugate Solution Conc. (100x)**

Prepare the working Conjugate Solution by adding 1 part Conjugate Solution Concentrate (100x) with 99 parts Conjugate Diluent. Example: 10 µl of Conjugate Solution Concentrate (100x) + 990 µl of Conjugate Diluent for 1 strip (8 wells).

Stability and storage: Opened Conjugate Solution Concentrate (100x) is stable 3 months when stored at 2-8°C. The working Conjugate Solution is stable 1 week when stored at 2-8°C.

## **Wash Solution Conc. (10x)**

Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 100 ml of Wash Solution Concentrate (10x) + 900 ml of distilled water for use of all 96-wells.

Stability and storage: The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

# Product Manual

## PREPARATION OF SAMPLES

The kit measures soluble HLA-G in EDTA plasma, amniotic fluid or culture supernatant. Samples should be assayed immediately after collection or should be stored at -20°C. Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Two diluents are available in the kit. Dilution Buffer 1 corresponds to the original version of the Dilution Buffer (the sole diluent present in the kit up to May 2014, and it is recommended for preparation of amniotic fluid samples. Additionally, Dilution Buffer 2 has been included starting from June 2014. This new diluent enables users to dilute EDTA plasma 8-fold and, thus, to further suppress potential matrix effects. Consequently, higher signal and better analytical characteristics are reached for EDTA plasma samples when using this new buffer.

### **EDTA plasma samples:**

Dilute samples 8x with Dilution Buffer 2 just prior to running the assay, e.g. 15 ml of sample + 105 ml of Dilution Buffer 2 for singlets, or preferably, 30 ml of sample + 210 ml of Dilution Buffer 2 for duplicates. Mix well (not to foam). Vortex is recommended.

### **Amniotic fluid samples:**

Dilute samples 4x with Dilution Buffer 1 just prior to the assay, e.g. 30 µl of sample + 90 µl of Dilution Buffer 1 for singlets, or preferably 60 µl of sample + 180 µl of Dilution Buffer 1 for duplicates. Mix well (not to foam). Vortex is recommended.

Stability and storage: Samples should be stored at -20°C, or preferably at -70°C for long-term storage. **Do not store the diluted samples.**

**Note:** *It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.*

## ASSAY PROCEDURE

1. Pipet 100 µl of Calibrators, samples and Dilution Buffer (= blank), preferably in duplicates, into the appropriate wells. See Figure 1 for example of work sheet.
2. Incubate the plate at 2-8°C for 16-20 hours, without shaking.
3. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add 100 µl of Conjugate Solution into each well.
5. Incubate the plate at room temperature (ca. 25°C) for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add 100 µl of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
8. Incubate the plate for 25 minutes at room temperature. The incubation time may be extended [up to 30 minutes] if the reaction temperature is below than 20°C. No shaking!
9. Stop the color development by adding 100 µl of Stop Solution.
10. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 – 650nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm. The absorbance should be read within 5 minutes following step 9.

**Note:** *If some samples and calibrator/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new calibrator curve, constructed using the values measured at 405nm, is used to determine soluble HLA-G concentration of off-scale calibrators and samples. The readings at 405 nm should not replace the readings for samples that were “in range” at 450 nm.*

**Note 2:** *Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.*

# Product Manual

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	Calibrator 125	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
B	Calibrator 62.5	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
C	Calibrator 31.25	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
D	Calibrator 15.63	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
E	Calibrator 7.81	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
F	Calibrator 3.91	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39
G	Blank	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40
H	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33	Sample 41

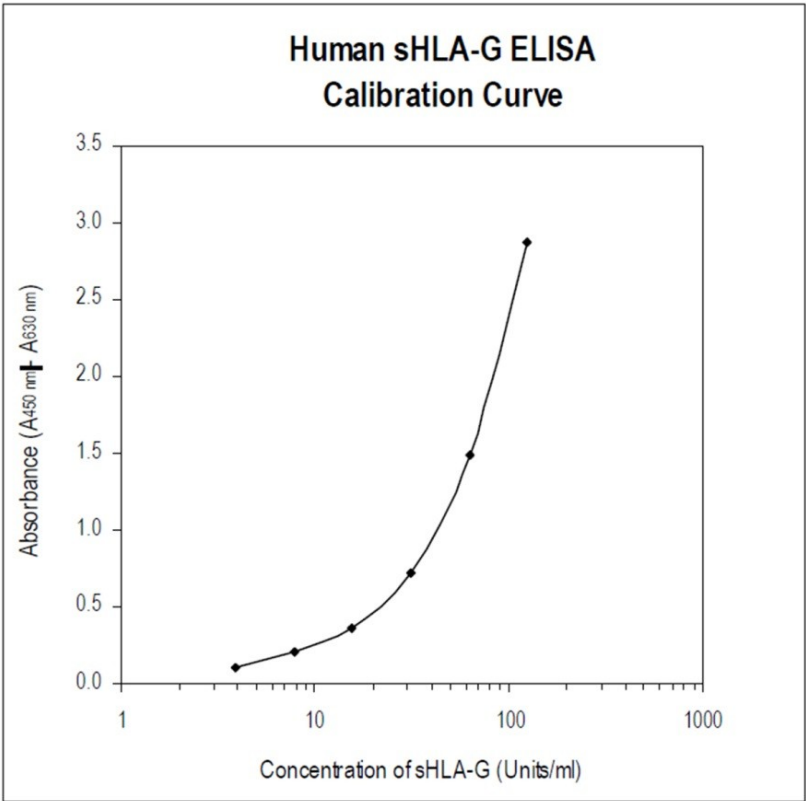
Figure 1: Example of a work sheet.

## CALCULATIONS

Most microplate readers perform automatic calculations of analyte concentration. The calibration curve is constructed by plotting the mean absorbance (Y) of Calibrators against the known concentration (X) of Calibrators in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of soluble HLA-G Units/ml in samples.

Alternatively, the logit log function can be used to linearize the calibration curve, i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of Calibrators.

**The measured concentration samples calculated from the calibration curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay, e.g. 25 U/ml (from calibration curve) x 4 (dilution factor) = 100 U/ml.**



**Figure 2:** Typical Calibration Curve for soluble HLA-G ELISA.

## PERFORMANCE CHARACTERISTICS

Typical analytical data of Novus Biologicals Human soluble HLA-G ELISA are presented in this chapter.

### Sensitivity

Limit of Detection (LOD), defined as concentration of analyte giving absorbance higher than mean absorbance of blank\* plus three standard deviations of the absorbance of blank:  $A_{\text{blank}} + 3 \times \text{SD}_{\text{blank}}$ , is calculated from the real soluble HLA-G values in wells and is 3 Units/ml.

\*Dilution Buffer is pipetted into blank wells.

### Limit of assay

Samples with absorbances exceeding the absorbance of the highest standard should be measured again with higher dilution. The final concentration of samples calculated from the standard curve must be multiplied by the respective dilution factor.

***Presented results are multiplied by respective dilution factor***

### Precision

Intra-assay (Within-Run) (n=8) for EDTA plasma samples diluted using the Dilution Buffer 2:

Sample	Mean (Unit/ml)	SD (Unit/ml)	CV (%)
1	327.75	16.01	4.9
2	658.77	23.02	3.5

Inter-assay (Run-to-Run) (n=5) for EDTA plasma samples diluted using the Dilution Buffer 2:

Sample	Mean (Unit/ml)	SD (Unit/ml)	CV (%)
1	342.31	22.32	6.5
2	909.63	67.39	7.4



## Spiking Recovery

EDTA plasma samples were spiked with different amounts of soluble HLA-G and assayed (Dilution Buffer 2 was used as a diluent).

Sample	<u>Observed</u> (Unit/ml)	<u>Expected</u> (Unit/ml)	Recovery O/E (%)
1	99.22		
	151.19	159.45	94.8
	191.28	216.77	88.2
	275.21	336.80	81.7
2	211.45		
	316.31	329.00	96.1
	730.01	449.03	95.8
	620.35	686.54	90.4

## Linearity

EDTA plasma samples were serially diluted with Dilution Buffer 2 and assayed.

Sample	Dilution	<u>Observed</u> (Unit/ml)	<u>Expected</u> (Unit/ml)	Recovery O/E (%)
1	-	175.59		
	2x	93.30	87.80	106.3
	4x	44.65	43.90	101.7
2	-	271.78		
	2x	136.16	135.89	100.2
	4x	60.74	67.95	89.4

## Stability of samples stored at 2-8°C

A significant decline in concentration of soluble HLA-G was observed in EDTA plasma after 7 days when stored at 2-8°C. Therefore, we strongly recommend storing the samples at -20°C, or preferably at -70°C for long-term storage.

## Effect of Freezing/Thawing

Significant changes in concentration of soluble HLA-G were observed in EDTA plasma samples after repeated freeze/thaw cycles. Therefore, it is strongly recommended to avoid unnecessary repeated freezing/thawing of the samples.



# Product Manual

Sample	Number of f/t cycles	EDTA Plasma (Unit/ml)
1	1x	134.64
	3x	233.28
	5x	206.72
2	1x	242.80
	3x	370.24
	5x	436.40
3	1x	625.60
	3x	860.80
	5x	890.80

### Reference Range

It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological reference ranges for soluble HLA-G levels with the assay.

### METHOD COMPARISON

The Human soluble HLA-G ELISA kit has not been compared to any other immunoassay.

### TROUBLESHOOTING AND FAQs

#### Weak signal in all wells

Possible explanations:

- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper wavelength when reading absorbance

#### High signal and background in all wells

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30°C

# Product Manual

## High coefficient of variation (CV)

### Possible explanation:

- Improper or inadequate washing
- Improper mixing Calibrators, Quality Controls or samples

## REFERENCES

### References to soluble HLA-G:

1. Rebman V., Pfeiffer K., Passler M., Ferrone S., Maier S., Weiss E., Grosse-Wilde H.: Detection of soluble HLA-G molecules in plasma and amniotic fluid. *Tissue Antigens*. 1999; 53: 14-22
2. Fuzzi B., Rizzo R., Criscuoli L., Noci I., Melchiorri L., Scarselli B., Bencini E., Menicucci A., Baricordi O.R.: HLA-G expression in early embryos is a fundamental prerequisite for the obtainment of pregnancy. *Eur J Immunol*. 2002; 32: 311-315
3. Lila N., Amrein C., Guillemain R., Chevalier P., Latremouille C., Fabiani J.N., Dausset J., Carosella E.D., Carpentier A.: Human leukocyte antigen-G expression after heart transplantation is associated with a reduced incidence of rejection. *Circulation*. 2002; 105: 1949-1954
4. Steinborn A., Rebmann V., Scharf A., Sohn C., Grosse-Wilde H.: Placental abruption is associated with decreased maternal plasma levels of soluble HLA-G. *J Clin Immunol*. 2003; 23: 307-314
5. Le Bouteiller P., Pizzato N., Barakonyi A., Solier C.: HLA-G, pre-eclampsia, immunity and vascular events. *J Reprod Immunol*. 2003; 59: 219-234
6. Sher G., Keskindepe L., Nouriani M., Roussev R., Batzofin J.: Expression of sHLA-G in supernatants of individually cultured 46-h embryos: a potentially valuable indicator of 'embryo competency and IVF outcome. *Reprod Biomed Online*. 2004; 9: 74-78
7. Noci I., Fuzzi B., Rizzo R., Melchiorri L., Criscuoli L., Dabizzi S., Biagiotti R., Pellegrini S., Menicucci A., Baricordi O.R.: Embryonic soluble HLA-G as a marker of developmental potential in embryos. *Hum Reprod*. 2005; 20: 138-146
8. Yie S.M., Balakier H., Motamedi G., Librach C.L.: Secretion of human leukocyte antigen-G by human embryos is associated with a higher in vitro fertilization pregnancy rate. *Fertil Steril*. 2005; 83: 30-36

# Product Manual

9. Rebman V., Lemaoult J., Rouas-Friess N., Carosella E.D., Grosse-Wilde H.: Report of the Wet Workshop for Quantification of Soluble HLA-G in Essen, 2004. *Hum Immunol.* 2005; 66: 853-863
10. Le Rond S., Azema C., Krawice-Radanne I., Durrbach A., Guettier C., Carosella E.D., Rouas-Freiss N.: Evidence to support the role of HLA-G5 in allograft acceptance through induction of immunosuppressive/regulatory T cells. *J Immunol.* 2006; 176: 3266-3276
11. Luque J., Torres M.I., Aumente M.D., Marin J., Garcia-Jurado G., Gonzalez R., Pascual D., Guerra N., Lopez-Rubio F., Alvarez-Lopez M.R., Arizon J.M., Pena J.: Soluble HLA-G in heart transplantation: their relationship to rejection episodes and immunosuppressive therapy. *Hum Immunol.* 2006; 67: 257-263
12. Carosella E.D., Favier B., Rouas-Freiss N., Moreau P., Lemaoult J.: Beyond the increasing complexity of the immunomodulatory HLA-G molecule. *Blood.* 2008; 111(10):4862-70
13. Apps R., Gardner L., Moffett A.: A critical look at HLA-G. *Trends Immunol.* 2008; 29(7):313-21
14. Baricordi O.R., Stignani M., Melchiorri L., Rizzo R.: HLA-G and inflammatory diseases. *Inflamm Allergy Drug Targets.* 2008; 7(2):67-74

## References to this product:

1. Wiendl H., Feger U., Mittelbronn M., Jack C., Schreiner B., Stadelmann C., Antel J., Brueck W., Meyermann R., Baror A., Kieseier B.C., Weller M.: Expression of the immune-tolerogenic major histocompatibility molecule HLA-G in multiple sclerosis: implications for CNS immunity. *Brain.* 2005; 128(Pt 11): 2689-2704
2. Desai N., Filipovits J., Singh P., Beman M., Goldfarb J.: Assessment of soluble HLA-G levels in day 3 human embryo culture media using a new ELISA kit: Is there a relationship to embryo quality, development and implantation potential? *Fertility & Sterility.* 2005; 84, Suppl 1 (S281-S282)
3. Steinborn A., Varkonyi T., Scharf A., Bahlmann F., Klee A., Sohn C.: Early detection of decreased soluble HLA-G levels in the maternal circulation predicts the occurrence of preeclampsia and intrauterine growth retardation during further course of pregnancy. *Am J Reprod Immunol .* 2007; 7(4):277-86
4. Tahan F., Patiroglu T. Plasma soluble human leukocyte antigen G levels in asthmatic children. *Int Arch Allergy Immunol .* 2006; 141(3):213-6 (2006)

# Product Manual

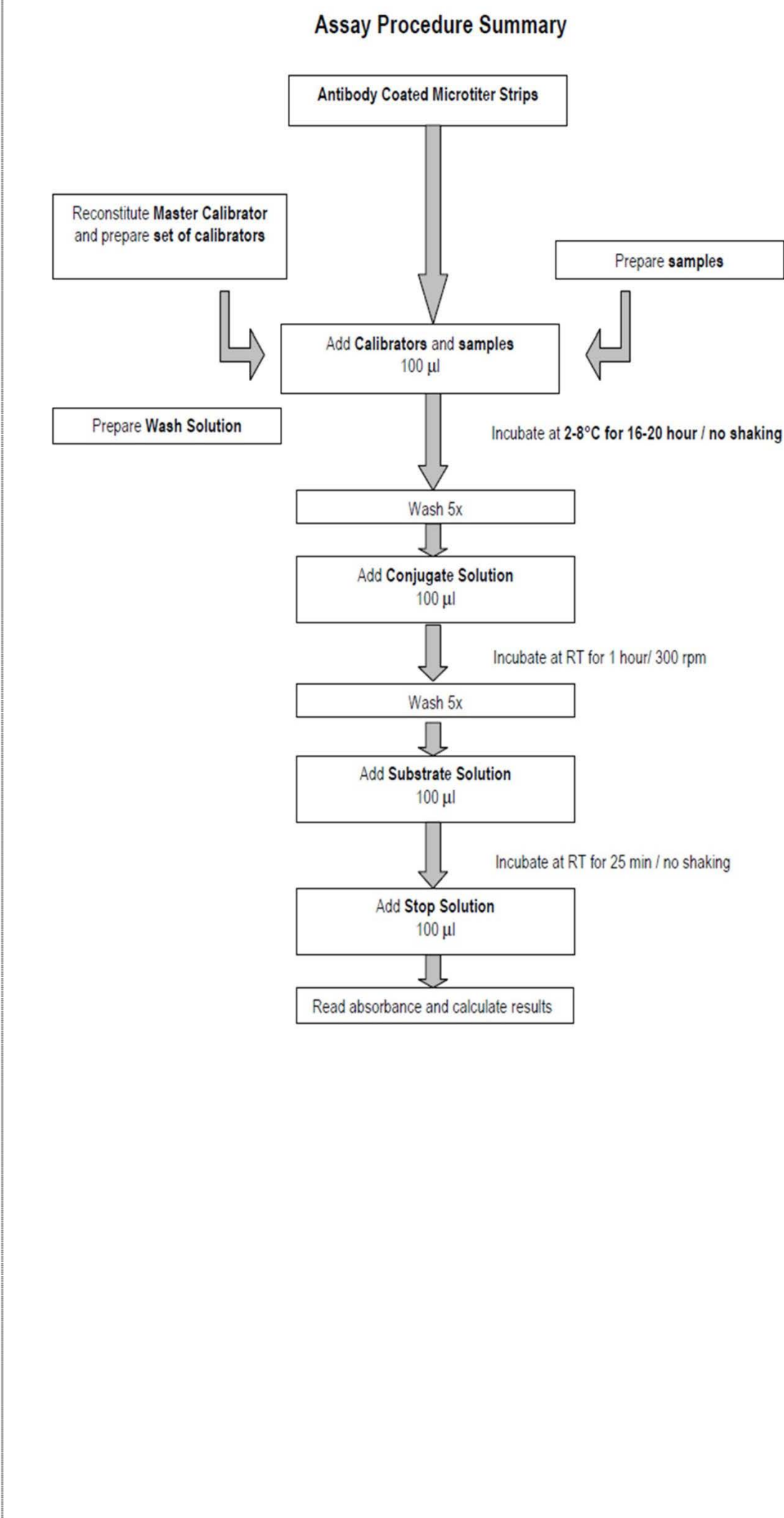
5. Desai N., Filipovits J., Goldfarb J.: Secretion of soluble HLA-G by day 3 human embryos associated with higher pregnancy and implantation rates: assay of culture media using a new ELISA kit. *Repro Biomed Online* . 2006; 13(2):272-7
6. Coulam C.B., Roussev R.G.: Soluble HLA G concentrations in culture media from in vitro fertilized human embryos are predictive of implantation potential. *American Journal of Reproductive Immunology*. 2005; 53:279-309
7. Wang F., Wen Z., Li H., Yang Z., Zhao X., Yao X.: Human leukocyte antigen-G is expressed by the eutopic and ectopic endometrium of adenomyosis. *Fertil Steril*. 2008; 90(5):1599-604
8. Shaikly V.R., Morrison I.E., Taranissi M., Noble C.V., Withey A.D., Cherry R.J., Blois S.M. and Fernández N.: Analysis of HLA-G in maternal plasma, follicular fluid, and preimplantation embryos reveal an asymmetric pattern of expression. *J Immunol*. 2008; 180(6):4330-7
9. Chen X.Y., Yan W.H., Lin A., Xu H.H., Zhang J.G., Wang X.X.: The 14 bp deletion polymorphisms in HLAG gene play an important role in the expression of soluble HLA-G in plasma. *Tissue Antigens*. 2008; 72(4):335-41
10. Ciprandi G., Colombo B.M., Contini P., Cagnati P., Pistorio A., Puppo F., Murdaca G.: Soluble HLA-G and HLA-A,-B,-C serum levels in patients with allergic rhinitis. *Allergy*. 2008; 63(10):1335-8
11. Eidukaite A., Tamosiunas V.: Soluble HLA-G in the peritoneal fluid of women with endometriosis. *Fertil Steril*. 2008; 89(2):465-7
12. Le Maux A., Noël G., Birebent B., Grosset J.M., Vu N., De Guibert S., Bernard M., Semana G., Amiot L: Soluble human leucocyte antigen-G molecules in peripheral blood haematopoietic stem cell transplantation: a specific role to prevent acute graft-versus-host disease and a link with regulatory T cells. *Clin Exp Immunol*. 2008; 152(1):50-6
13. Giannopoulos K., Schmitt M., Kowal M., Własiuk P., Bojarska-Junak A., Roliński J., Dmoszyńska A.: The significance of soluble HLA-G plasma levels as well as messenger HLA-G for B-cell chronic lymphocytic leukemia (B-CLL). *Leuk Res*. 2008; 32(12):1815-9
14. McCormick J., Whitley G.S., Le Bouteiller P., Cartwright J.E.: Soluble HLA-G regulates motility and invasion of the trophoblast-derived cell line SGHPL-4. *HumReprod*. 2009; 24(6):1339-45

# Product Manual

15. Kusanovic J.P., Romero R., Jodicke C., Mazaki-Tovi S., Vaisbuch E., Erez O., Mittal P., Gotsch F., Chaiworapongsa T., Edwin S.S., Pacora P., Hassan S.S.: Amniotic fluid soluble human leukocyte antigen-G in term and preterm parturition, and intra-amniotic infection/inflammation. *J Matern Fetal Neonatal Med.* 2009; 22(12):1151-66
16. Roussev R.G., Acacio B., Parrett S., Romineb H., Coulam C.B.: Soluble HLA-G as potential candidate to help morphology grading score in embryo selection for IVF/ET. *Journal of Reproductive Immunology.* 2009; 81, 113–175
17. Ivanova-Todorova E., Mourdjeva M., Kyurkchiev D., Bochev I., Stoyanova E., Dimitrov R., Timeva T., Yunakova M., Bukarev D., Shterev A., Tivchev P., Kyurkchiev S.: HLA-G expression is up-regulated by progesterone in mesenchymal stem cells. *Am J Reprod Immunol.* 2009; 62(1):25-33
18. Mach P., Blecharz P., Basta P., Marianowski P., Skret-Magierlo J., Kojs Z., Grabiec M., Wicherek L.: Differences in the soluble HLA-G blood serum concentration levels in patients with ovarian cancer and ovarian and deep endometriosis. *Am J Reprod Immunol.* 2010; 63(5):387-95
19. Chen C.H., Liao H.T., Chen H.A., Liu C.H., Liang T.H., Wang C.T., Tsai C.Y., Chou C.T.: Human leukocyte antigen-G in ankylosing spondylitis and the response after tumour necrosis factor-alpha blocker therapy. *Rheumatology (Oxford).* 2010; 49(2):264-70

# Product Manual

## ASSAY PROCEDURE SUMMARY



# Product Manual

1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
A								
B								
C								
D								
E								
F								
G								
H								

# Product Manual

