PAIA*manual*

Human IgG Fc/Fab medium titer assay kit
PA-101-01 (1 kit) or PA-101-10 (10 kits)

Contents

Introduction

Objective

The present kit is designed for the rapid quantification of human Immunoglobulins G (IgGs) in sample volumes of 2 - 10 µL for applications like high-producer screening in cell line development. It contains a 384-well PAIA*plate* and reaction buffer with a fluorescent marker sufficient for generating 384 data points.

PAIA Technology

PAIA*plates* have protrusions on the bottom of each well that allow settling of capture beads in the area which is not accessible to fluorescence read-out (Figure 1A). The assays contain functionalized beads to capture IgG and a fluorescent marker for detection. During incubation, the analyte IgG is bound by Protein A beads and fluorescent marker, forming bead-analyte-marker complexes (Figure 1B, on the right). While Protein A interacts with the Fc part of IgG, the fluorescence marker of this kit targets the $F(ab')_2$ region.
After incubation and bead settling, unbound marker is measured through the transparent protrusions which keep the beads outside the detection zone and light path. Thus, PAIA assays measure the fluorescence of unbound marker remaining in solution.

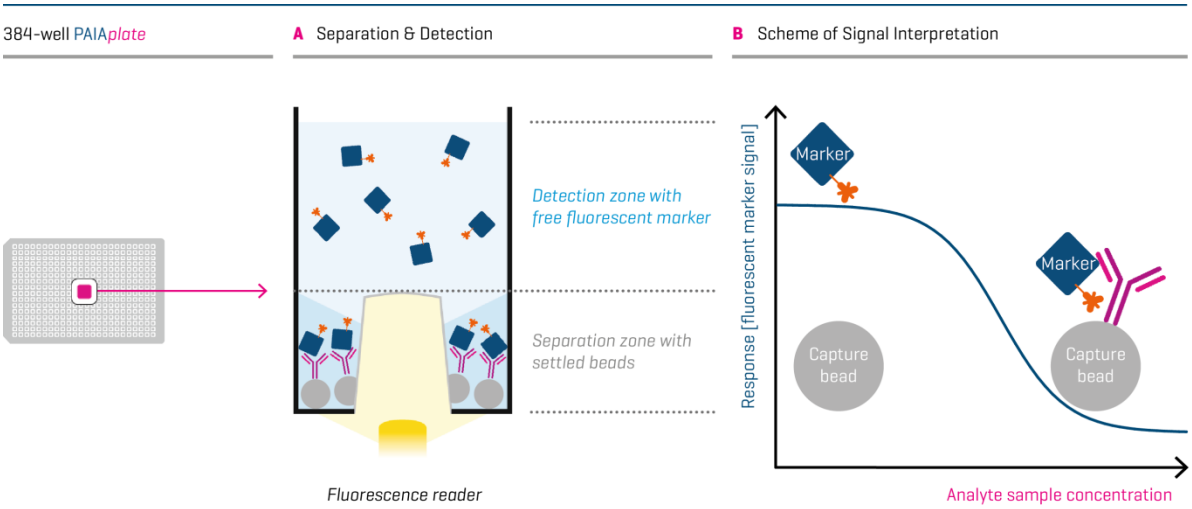


Figure 1: The design of the wells of the PAIA*plates* and the assay components. A) After incubation and bead separation, unbound fluorescent marker is measured through the transparent protrusions. B) The IgG is bound by Capture Beads and fluorescent marker, forming bead-analyte marker complexes resulting in a decrease of fluorescence signal with increasing analyte concentration.

Analyte of Interest

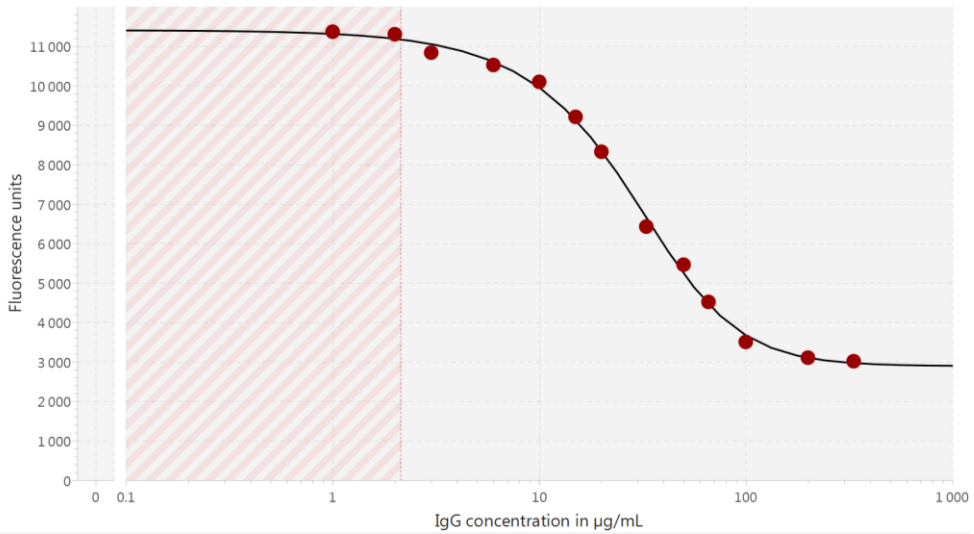
Immunoglobulin G antibodies have a four-chain structure, comprising two identical heavy (H) chains and two identical light (L) chains, linked together by inter-chain disulfide bonds to a Y-shape. Four IgG subclasses have been identified; they differ mainly in the molecular weight of their heavy chains and the number of disulfide bonds in the hinge region.
The PAIA human IgG Fc/Fab kit recognizes all human IgG proteins that interact with Protein A namely IgG1, IgG2, and IgG4. While Protein A interacts with the Fc part of IgG, the fluorescent marker of this kit targets the $F(ab')_2$ region. IgG fragments lacking either the Fc or the Fab part will not be detected.

Assay Performance Characteristics

The PAIA human IgG Fc/Fab kit was optimized for high-producer screenings. The standard protocol covers the relevant concentration range without the need to dilute samples further. The protocol can be modified in order to change the dynamic range and the Limit of Detection (LoD). This can be achieved by increasing or decreasing the sample volume.

For the standard protocol (sample volume of 6 µL in 60 µL total assay volume) the performance criteria are specified as:

- Sensitivity: The LoD is in the range of 2-4 µg/mL.
- Validated concentration range: 5 – 200 µg/mL (according to the EMEA guideline for bioanalytical method validation)
- A representative calibration curve with human IgG1 is shown below.



Date: 2016-10-13 15:05	Determination (R^2): 0.9972	Minimum (A): 2 889.17
Model: 5-Param. Logistic	LoD: 2.15	Slope (B): −2.03
	LoQ: 4.92	Inflection (C): 42.79
		Maximum (D): 11 403.09
		Asymmetry (E): 0.5888

Kit Contents

Reagent	Quantity	Storage
PAIA*plate*	One 384-well plate containing desiccated capture beads	Store at 2 - 8°C. Do not remove the wrapping until usage. Stable for 3 months. Once opened, the plate is stable for 2 months when resealed and stored dry and in its wrapping at 2 - 8°C.
PAIA*mix*	30 mL brown bottle containing buffer with fluorescent marker	Store at 2 - 8°C. Stable for 3 months.

Note: the calibration standard is not included in this kit.

Quality Control

Assay performance is tested for each lot of assay kits according to the standard protocol described herein. A certificate of analysis (COA) for each assay lot is available on request.

Equipment required

- Pipetting system (ideally multichannel, automated) for the 2 - 10 µL and 50 - 100 µL range.
- Orbital microplate shaker. The optimal shaker speed is 1800 rpm for shakers with an orbit of 2 mm and 1400 rpm for shakers with 3mm orbit. Slower shakers can also be used but require significantly longer incubation times.
- Fluorescence microplate reader with bottom reading options and suited for 384-well formats (e.g. from Tecan, Molecular Devices and BMG Labtech) or automated fluorescence microscopes, (e.g. Cellavista / NyOnNE from SynenTec).
- Optional: microplate centrifuge

Precautions

- Please read these instructions carefully prior to beginning the assay. Modifications to the kit components or procedures may result in loss of performance.
- Please keep the PAIA*mix* in its original bottle at 2 - 8°C.
- The PAIA assay is intended for research purposes only and is not for use in diagnostic procedures.

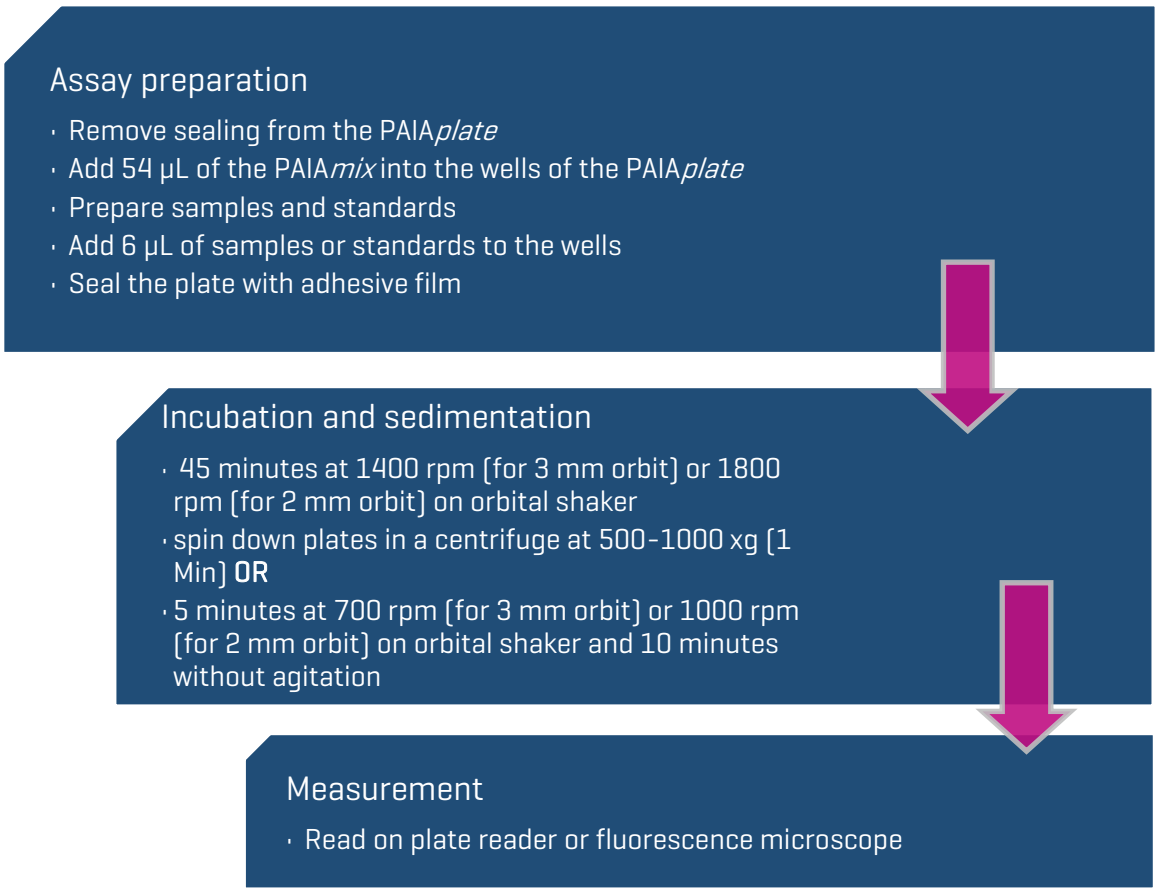
Technical Recommendations

- Data quality depends on the read-out instrument and its configuration. Accurate plate positioning for the 384-well plate is required. The dimensions of the PAIA*plate* are similar to the Greiner 384-well plate format (greiner384ft) which is available as a template in the software of most plate readers. Therefore, it is advisable to optimize the plate definition settings once before getting started. This is done by varying the parameters depicted below until the highest fluorescence intensity and lowest CVs are reached (e.g. in all four corner wells filled with PAIA*mix*).
- Change tips between sample, standard, and reagent additions to avoid cross contamination of samples or reagents.
- When reagents are added, make sure the liquids are at the bottom of the well. Avoid introduction of air bubbles.
- The PAIA signal can slightly vary with temperature and incubation time. For optimal results, identical incubation times and temperature should be used for standards and unknown samples.
- Efficient mixing of the PAIA*plate* is crucial for accurate results. If you are not sure about the mixing performance, optimize the parameters of the mixing / incubation step. In general, higher rpm numbers and longer incubation times improve assay performance. However, too strong agitation can result in spilling of samples.
- The standard curves shown in this technical data sheet are provided for information only. A standard curve should be generated for each experiment. The standard curve should be prepared in the same matrix as the samples.
- If you have only used part of the plate, please wrap the plate into the silver envelope and keep it in the fridge at 4° C. Please store the remaining PAIA*mix* in the fridge as well.

Assay Protocol

Workflow of the PAIA assay

For a video tutorial please go to: http://www.paiabio.com/technology/workflow/

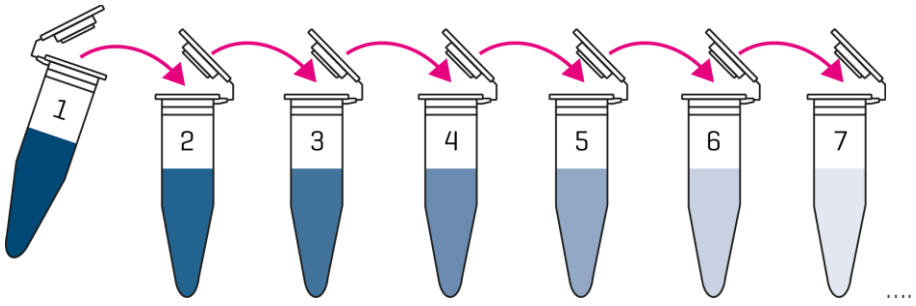


Standard Protocol

- Remove sealing tape from the PAIA*plate*.
- Add 54 µL of the ready-to-use PAIA*mix* into the wells of the PAIA*plate*.
- Prepare the human IgG standard by dilution of a standard protein of choice in the same matrix as your samples, e.g. mock cell culture supernatant, medium or buffer.

Note: The standards should cover the concentration range of 2 - 200 µg/mL. We recommend using at least 10 standards with at least two replicates to obtain good calibration data. E.g. prepare 105 µL of 200 µg/mL IgG solution. Dilute 1 : 1.4 by mixing 75 µL IgG with 30 µL matrix. Repeat 13 times to yield 30 µL of the following concentrations: 200 / 142.9 / 102.0 / 72.9 / 52.1 / 37.2 / 26.6 / 19.0 / 13.6 / 9.7 / 6.9 / 4.9 / 3.5 / 2.5 µg/mL. Do not forget to prepare negative controls containing only matrix.

Solution #	Concentration	Matrix	IgG	Dilution factor
1	200.0 µg/mL	-	105 µL	
2	142.9 µg/mL	30 µL	75 µL of 200.0 µg/mL solution	1.4
3	102.0 µg/mL	30 µL	75 µL of 142.9 µg/mL solution	1.4
4	72.9 µg/mL	30 µL	75 µL of 102.0 µg/mL solution	1.4
5	52.1 µg/mL	30 µL	75 µL of 72.9 µg/mL solution	1.4
6	37.2 µg/mL	30 µL	75 µL of 52.1 µg/mL solution	1.4
7	26.6 µg/mL	30 µL	75 µL of 37.2 µg/mL solution	1.4
8	19.0 µg/mL	30 µL	75 µL of 26.6 µg/mL solution	1.4
9	13.6 µg/mL	30 µL	75 µL of 19.0 µg/mL solution	1.4
10	9.7 µg/mL	30 µL	75 µL of 13.6 µg/mL solution	1.4
11	6.9 µg/mL	30 µL	75 µL of 9.7 µg/mL solution	1.4
12	4.9 µg/mL	30 µL	75 µL of 6.9 µg/mL solution	1.4
13	3.5 µg/mL	30 µL	75 µL of 4.9 µg/mL solution	1.4
14	2.5 µg/mL	30 µL	75 µL of 3.5 µg/mL solution	1.4
15	Negative control	30 µL	-	-



- Add 6 µL of standard/sample into the PAIA*mix* -containing wells.
- Seal the plate with adhesive film
- Incubate the plate on the orbital shaker for **45 minutes at 1400 rpm (for 3 mm orbit) or 1800 rpm (for 2 mm orbit) on orbital shaker**
- After the incubation the beads need to settle outside the detection zone. Therefore, either spin down the beads on a microplate centrifuge at 500-1000 xg for 1 minute **OR** reduce the shaking speed on the orbital shaker to **700 rpm (for 3 mm orbit) or 1000 rpm (for 2 mm orbit)** and keep shaking the plate for 5 minutes.

- Then, let the plate stand without movement for another 10 minutes. You can omit this step if you have used a centrifuge to spin down the beads.

Measurement

PAIA*plates* can be measured with fluorescence plate readers or automated fluorescence microscopes. Both yield comparable data quality. The read-out time is also comparable: 3 - 5 minutes per plate. Individual performance depends on the read-out system and it´s fit with the PAIA*plate*.

Please make sure that the calibration standards fit well in the dynamic range of the instrument and avoid saturation of the detector. Sensitivity is no issue with standard readers.

Correct plate definition is important to ensure optimal reading of the PAIA*plate*.

Measurement with a plate reader:

- When using a fluorescence plate reader the bottom read mode has to be selected.
- Excitation wavelength: 640 nm; Emission wavelength: 665 nm
- The detection parameters should be optimized once (excitation time, lamp energy, number of flashes, gain etc.) to ensure that the generated signals fit well into the dynamic range of the reader.

Measurement with a fluorescence microscope:

- The fluorescence microscope must be capable of imaging the well center and to measure the intensity. We recommend taking a single image with a 10x or 20x objective at a defined distance to be determined by the user.
- Filter settings for dyes like Cy5, Alexa 647, e.g. for Cellavista and NyONE instruments: excitation source: red / emission filter: deep red.
- Users of Cellavista or NyONE imagers from SynenTec can use the PAIA widget in the instrument software. If this is not available, please update the instrument software.
- Optimization of the plate definition and microscope settings (excitation time and energy, camera gain etc.) are done by inspection of the images.

Data Analysis

Data analysis can be performed with any software capable of generating calibration curves with 4 or 5 parameter fits and calculating concentrations of unknown samples. PAIA Biotech offers a software tool which is easy to operate and which is compatible with a large range of reader types.

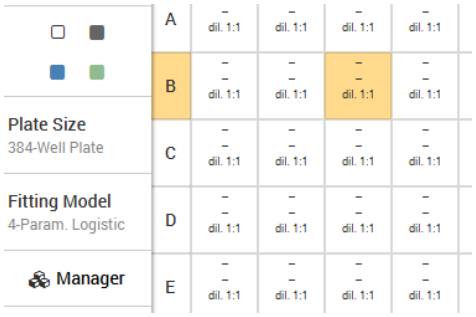
PAIA Evaluation tool

The PAIA Evaluation tool is a clearly arranged, stand-alone program optimized for analyzing PAIA assays that can be run on any PC with JAVA installed. Raw data from different sources can be imported / copied and processed easily.

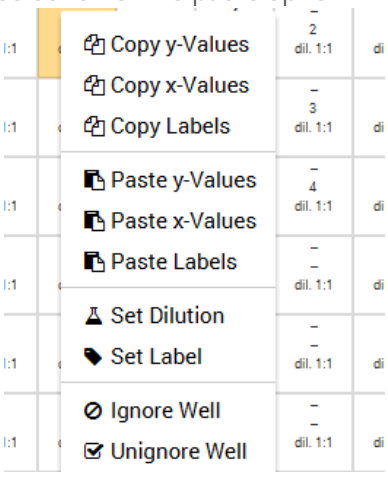
The PAIA Evaluation tool and the manual for the tool can be obtained from PAIA Biotech (email to contact@paiabio.com).

Definition of a plate layout and insertion of data

- Choose the plate size (384-wells) and define each well using the colored buttons in the upper part of the menu panel on the left. You can choose between empty well (white), background well (grey), calibration well (blue), and unknown sample well (green).
- If you intend to use the same layout several times you can store it using the Manager function.

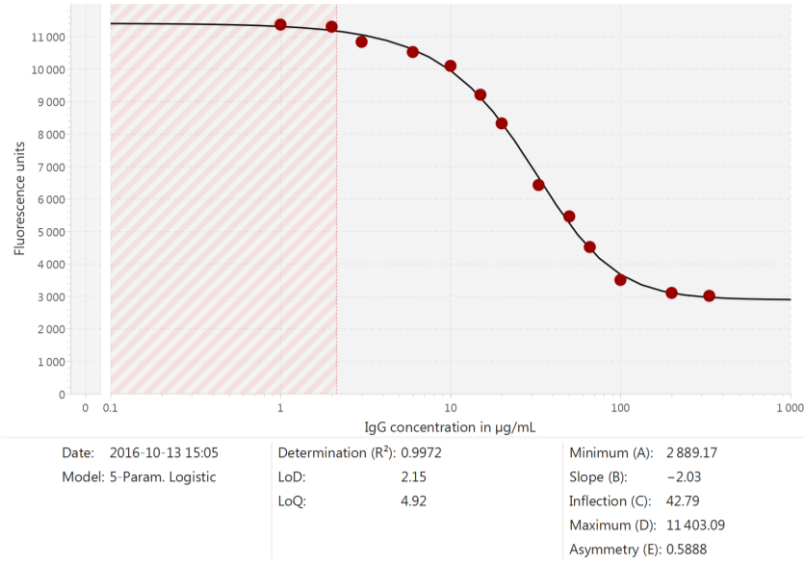


- With the right mouse button a menu opens for several actions:
  - Each well can be marked with a label and its dilution.
  - Measurement data and concentration of the calibration samples can be pasted into the input layout by selection of the paste option.



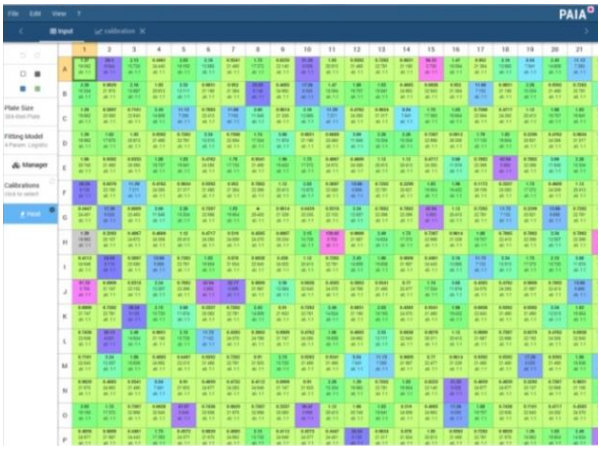
Generation of calibration curves

- Calibration curves are generated by using the path: Edit\Fit Calibration Group or the shortcut Ctrl+f
- Calibration curves are shown on a separate page. The calibration data can be viewed and the graph can be designed according to the desired layout. Please use the left menu panel in order to
    - Rename the calibration graph or the axes of the graph.
    - Display the y-axis with absolute (Abs.) or normalized (Rel.) values; the x-axis can be depicted as linear (Linear) or logarithmic (Log.) scale.
    - Estimate the quality by selecting the LOD or LOQ view.
- A picture of the finalized graph can be exported by the screenshot function.
- The information on the fitting model as well as the obtained constants are depicted below the graph, they can be included in the screenshot if desired.



Generation of results and export of data

Once a calibration curve has been obtained, the concentration of the unknown samples can be calculated. Heat maps show the results at one glance, including dilution factors. Several export functions are available for export and reporting.



Troubleshooting

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Prepare fresh calibration standards
	Cross contamination	Change pipette tips between pipetting steps
Large CV	Inaccurate pipetting	Check pipettes
	Insufficient incubation	Check speed of the shaker, increase incubation time
	Evaporation, especially in the exterior wells	Seal or cover plates during incubation
Low negative control values	Inadequate reagent volumes added to wells	Check pipettes
	Inadequate storage of PAIA*mix*	Store at 2°C - 8°C
High negative control values	Evaporation	Seal or cover plates during incubation
PAIA Evaluation tool yields no concentration values	Values of samples outside dynamic range	Dilute samples if concentration is too high or use larger volumes if concentration is too low

For support inquiries please phone us at +49-(0) 221 16862380 or email to contact@paiabio.com

Please also check the FAQ section on paiabio.com

Related products

Article No.	Description	Content
PA-101-**01**	Human IgG Fc/Fab medium titer assay kit	1 kit
PA-101-**10**	Human IgG Fc/Fab medium titer assay kit	10 kits
PA-102-**01**	Human IgG Fc/Fab low titer assay kit	1 kit
PA-102-**10**	Human IgG Fc/Fab low titer assay kit	10 kits
PA-103-**01**	Fc medium titer assay kit	1 kit
PA-103-**10**	Fc medium titer assay kit	10 kits
PA-104-**01**	Fc low titer assay kit	1 kit
PA-104-**10**	Fc low titer assay kit	10 kits
PA-105-**01**	Human Fab medium titer assay kit	1 kit
PA-105-**10**	Human Fab medium titer assay kit	10 kits

Purchaser Notification

Limited Use Statement

The Human IgG Fc/ Fab medium titer kit was developed at PAIA Biotech GmbH. PAIA Biotech has filed a patent application for the technology used in this assay. The purchase of this product conveys to the buyer the non-transferable right to use the purchased product in research conducted by the buyer. The buyer cannot sell or otherwise transfer this product or any component thereof to a third party or otherwise use this product or its components for commercial purposes. Commercial purposes include, but are not limited to: use of the product or its components in manufacturing and resale of the product or its components. The terms of this Limited Use Statement apply to all buyers including academic and for-profit entities. If the purchaser is not willing to accept the conditions of this Limited Use Statement, PAIA Biotech GmbH is willing to accept return of the unused product with a full refund.

Limited Warranty

PAIA Biotech GmbH warrants that, at the time of shipment, the products sold by it are free from defects in material and workmanship and conform to specifications. PAIA Biotech GmbH makes no other warranty, express or implied with respect to the products, including any warranty of merchantability or fitness for any particular purpose. Notification of any breach of warranty must be made within 60 days of receipt unless otherwise provided in writing by PAIA Biotech GmbH. No claim shall be honored if the customer fails to notify PAIA Biotech GmbH within the period specified. The sole and exclusive remedy of the customer for any liability PAIA Biotech GmbH of any kind including liability based upon warranty (express or implied whether contained herein or elsewhere), strict liability contract or otherwise is limited to the replacement of the goods or the refunds of the invoice price of goods. PAIA Biotech GmbH shall not in any case be liable for special, incidental or consequential damages of any kind.

PAIA Biotech GmbH
Gottfried-Hagen-Strasse 60-62
51105 Köln
Germany
Tel. +49-221-168623-80
www.paiabio.com