# Product information

# C5a ELISA

Enzyme immunoassay for the determination of Anaphylatoxin C5a in human plasma or urine

REF IB79153

Σ 192

RUO **For Research Use Only – Not for Use in Diagnostic Procedures**



Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave. NE, Suite P, Minneapolis, Minnesota 55432, USA
Phone: +1 (763) - 780-2955 Fax.: +1 (763) - 780-2988
Email: ibl@ibl-america.com Web: www.ibl-america.com

## Contents





Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave. NE, Suite P, Minneapolis, Minnesota 55432, USA
Phone: +1 (763) - 780-2955 Fax.: +1 (763) - 780-2988
Email: ibl@ibl-america.com Web: www.ibl-america.com

# 1 INTRODUCTION

## 1.1 Intended Use

The **IBL-America C5a ELISA** is an enzyme immunoassay for the determination of Anaphylatoxin C5a in human plasma or urine. For research use only, not for use in diagnostic procedures.

## 1.2 Summary and Explanation

The complement system consists of more than 20 proteins which evolved as defense system against invading microorganisms. It can also be activated in a variety of disease states or upon contact with medical devices or drugs (1). Upon activation, a cascade of proteolytic enzymes releases the anaphylatoxins C3a, C4a and C5a from their respective precursors (2). These fragments exert various biological functions such as histamine release, smooth muscle contraction, increase in capillary permeability or immunomodulation (3). In addition, C5a and its degraded form C5a-desArg are highly potent chemotactic agents for polymorphonuclear leukocytes, which then will release tissue degradative enzymes and oxygen radicals (4). This in turn will also lead to activation of other humoral systems such as coagulation and fibrinolysis (5). Thus, C5a is probably the most important complement-derived proinflammatory mediator. C5a is believed to play a pivotal role in the pathogenesis of septic shock, the adult respiratory distress syndrome, acute pancreatitis and the deleterious effects after myardial infarction (6,7,8). Recently it has been shown that C5a is closely associated with the capillary leak syndrome in leukemic children after bone marrow transplantation. C5a is also a marker in urine for predicting the onset of acute graft rejection after kidney transplantation (9). With respect to possible deleterious consequences, C5a determination may be indicated during hemodialysis, after cardiopulmonary bypass or after any other contact with medical devices (10).

# 2 PRINCIPLE OF THE TEST

The IBL-America C5a ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle.
Due to cross-reactivity of the monoclonal antibodies with complement factor C5, C5 in the sample is removed by precipitation prior to analysis. The resulting clear supernatant contains the C5a to be determined (10,11). During the first incubation the C5a in the sample binds to murine anti C5a monoclonal antibodies (mab 561), which are attached to the surface of the microtitration plate. Unbound constituents are then removed by washing and, in a second reaction, peroxidase conjugated monoclonal antibodies (Mab 557) are added and bound to a different epitope on C5a. The excess enzyme conjugated antibodies are removed by washing; the bound enzyme activity is then determined. The enzymatic reaction between hydrogen peroxide and chromogen is terminated by the addition of dilute acidic solution.
The intensity of the colour intensity, which is proportional to the concentration of C5a, is determined photometrically.


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## 3 WARNINGS AND PRECAUTIONS

1. For research use only, not for use in diagnostic procedures.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21 °C - 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where samples or kit reagents are handled.
12. Wear disposable latex gloves when handling samples and reagents. Microbial contamination of reagents or samples may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with *Stop Solution* containing 1 N acidic solution. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request directly from IBL-America.

## 4 REAGENTS

### 4.1 Reagents provided

1. ***Microtiterwells***, 24 x 8 (break apart) strips, 192 wells;
   Wells coated with murine monoclonal antibodies against human C5a.
2. ***Standard (Standard 1-4)***, 4 vials (lyophilized), 1.0 mL;
   Concentrations: 0.1 - 0.4 - 3.0 - 10.0 µg/L
   See “Reagent Preparation”;
3. ***Control***, 1 vial (lyophilized), 1.0 mL,
   see “Reagent Preparation”
   For control values and ranges please refer to vial label or QC-Datasheet.
4. ***Assay Buffer***, 1 vial, 25 mL, ready to use,
5. ***Enzyme Conjugate,*** concentrate, 1 vial, 0.5 mL,
   Murine monoclonal antibodies to human C5a, conjugated to horseradish peroxidase;
   see “Reagent Preparation”.
6. ***Conjugate Diluent***, 2 vials, 11 mL each, ready to use
   Tris Buffer solution (50 mmol/L)
7. ***Precipitation Reagent,*** 1 vial, 20 mL, ready to use,
8. ***Substrate Solution***, 1 vial, 25 mL, ready to use,
   Tetramethylbenzidine (TMB).
9. ***Stop Solution***, 1 vial, 25 mL, ready to use,
   contains 1 N acidic solution,
   Avoid contact with the stop solution. It may cause skin irritations and burns.
10. ***Wash Solution***, 1 vial, 30 mL (40X concentrated),
    see “Reagent Preparation”.

### 4.2 Materials required but not provided

- Tris Buffer Solution (Tris/HCl Buffer pH 8.0 Tris (100 mmol/L), NaCl (25 mmol/L). For sample dilution. (This solution can be ordered separately)
- Centrifuge: suited for small reaction tubes (e.g. Eppendorf)
- A microtiter plate calibrated reader (450 ± 10 nm)
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

### 4.3 Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2 °C to 8 °C. For special storage conditions please refer to chapter 4.4 “*Reagent Preparation*”.

Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for 4 weeks if stored as described above.

The Precipitation Reagent has to be stored protected from light.

### 4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

***Standards and Control***

Reconstitute the lyophilized contents of the standard vial with 1.0 mL deionized water.

***Note:*** *The reconstituted standards and control can be used within 8 hours at 15 °C to 25 °C°C or within 1 day at 2 °C to 8 °C.*
*For longer storage freeze at -20 °C for 4 weeks.*
*Frozen (-20°C) reconstituted Standards or Control should only be used once within 4 weeks.*

***Wash Solution***

Add deionized water to the 40X concentrated Wash Solution.
Dilute 30 mL of concentrated *Wash Solution* with 1170 mL deionized water to a final volume of 1200 mL.
*The diluted Wash Solution is stable for 2 weeks at room temperature.*

***Enzyme Conjugate***

Pipette 200 µL of Anti-human C5a Conjugate into a vial of Conjugate Diluent (11 mL) and shake gently to mix (sufficient for 1 test plate).
Working Conjugate Solution can be stored at 2 °C to 8 °C for 4 weeks.

**Dilution Reagent** (for Sample dilution)
Prepare a Dilution Reagent by mixing an equal volume of Tris buffer with distilled water.

### 4.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheet.

### 4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, IBL-America has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.


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## 5 SAMPLE COLLECTION AND PREPARATION

Plasma or urine can be used in this assay.
Haemolytic and lipaemic plasma and plasma containing rheumatoid factors do not interfere with the assay.
Please note: Samples containing sodium azide should not be used in the assay.

### 5.1 Sample Collection

**Plasma:**
Whole blood should be collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection. (E.g. for EDTA plasma Sarstedt Monovette – red cap - # 02.166.001;

Centrifuge within 2 hours for 10 min. at a minimum of 1500 x g and remove the supernatant plasma.
C5a is preferentially determined in plasma or urine stabilized with EDTA (≥ 10 mmol/L final concentration).
Citrated plasma may also be used but requires special care as e.g. immediate cooling at ice in order to avoid unspecific activation of the complement cascade (13).

**Urine**
In urine C5a is stable at room temperature (15 °C to 25 °C) for 24 hours (9).
Thus, urine routinely collected over 24 hours can be used as well as spontaneous urine. In case of severe proteinuria additional cleavage of excreted C5 might occur.
For collection of urine 1 part of an appropriate EDTA solution (> 0.11 nmol/L) is mixed with 9 parts of urine.

### 5.2 Sample Storage

| | | |
|---|---|---|
| Stability of **plasma** sample: | 15 °C – 25 °C | 2 hours |
| | 2 °C – 8 °C | 8 hours |
| | - 20 °C | 1 month |

Thawed samples should be inverted several times prior to testing.

| | | |
|---|---|---|
| Stability of **urine** sample: | 15 °C - 25 °C | 24 hours |

### 5.3 Sample Dilution

If in an initial assay, a sample is found to contain more than the highest standard or if high values are expected dilute the plasma sample first 1:10 with *Dilution Reagent* and then apply the normal precipitation step to the diluted sample.
For the calculation of the concentrations this dilution factor has to be taken into account.

Example dilution 1:10: 10 µL sample + 90 µL *Dilution Reagent* (mix thoroughly)

In the case that the amount of sample is limited the further dilution can be prepared from the supernatant. But for exact results, the sample dilution must be done before the precipitation step.

### 5.4 Preparation of Samples - Precipitation

In order to exclude cross-reactivity of the monoclonal antibodies with uncleaved complement factor C5, the C5 in standards, control plasma and samples has to be removed by precipitation. After centrifugation the clear supernatant contains the C5a to be analysed.

1. Pipette into appropriate centrifugation tubes one volume of either sample, standard or control plasma and add one volume of the Precipitation Reagent. For double determinations a volume of 100 µL of sample and 100 µL of Precipitation Reagent is recommended.
2. Mix intensively at once and incubate at least for 3 min. at 15 °C – 25 °C.
3. Centrifuge the mixture for 10 min. at approx. 2500 x g (or 3 min. at 8000 x g).
4. **Use the clear supernatant in the assay procedure.**

In the supernatant C5a is stable at 15 °C – 25 °C for 1 day and at 2 °C – 8 °C for 3 days if stored separately from the pellet.

# 6 ASSAY PROCEDURE

## 6.1 General Remarks

- All reagents and samples must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

## 6.2 Test Procedure

Each run must include a standard curve.

### 6.2.1 Assay Procedure for Plasma Samples

1. Secure the desired number of Microtiterwells in the holder.
2. Pipette into each well **50 µL** of Assay Buffer (C5a).
3. Dispense **50 µL** of the supernatant of either standard, control or sample **with new disposable tips** into appropriate wells. After filling the test plate shake briefly to ensure thorough mixing.
4. Incubate for **20 min**. ± 2 min.) at room temperature (20 °C – 25 °C).
5. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with diluted Wash Solution (300 µL per well). Strike the wells sharply on absorbent paper to remove residual water droplets.
   **Important note:**
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Dispense **100 µL** Working Conjugate Solution into each well.
7. Incubate for **15 min**. (± 2 min.) at room temperature (20 °C – 25 °C).
8. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with diluted Wash Solution (300 µL per well). Strike the wells sharply on absorbent paper to remove residual water droplets. (See step 5.)
9. Add **100 µL** of Substrate Solution to each well.
10. Incubate for **15 minutes** (± 2 min.) at room temperature.
11. Stop the enzymatic reaction by adding **100 µL** of Stop Solution to each well.
12. Determine the absorbance (OD) of each well at **450 ± 10 nm** with a microtiter plate reader.
    It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

### 6.2.2 Assay Procedure for Urine Samples

For testing urine samples the following procedure is recommended to obtain an improved recovery of C5a.

1. Instead of using Standards S1 to S4 for establishing the reference curve **use only standard S4** and prepare a precipitate as described.
2. Then dilute the supernatant in series (1:2, 1:4, 1:8, 1:16) with a 1:1 mixture of the Precipitation Reagent and the following buffer:
   150 mmol/L Na-phosphate, 150 mmol/L NaCl, 10 mmol/L EDTA, pH 7.0.
3. For dilution of urine samples follow the same procedure, i.e. dilute the supernatant after precipitation with the phosphate buffered saline/Precipitation Reagent mixture.
4. With this prepared Standard curve and urine samples follow now the procedure as described in “6.3.1 Assay Procedure for Plasma Samples”.

### 6.3 Results

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as such. For the calculation of the concentrations this dilution factor has to be taken into account.

## 7 EXPECTED NORMAL VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

Preliminary Reference Interval:
The Normal Values of the IBL-America ELISA were determined by measuring the Values of 240 apparently healthy adults with the IBL-America ELISA Kit.
The normal value range is assumed to be as $2.5^{th}$ – $97.5^{th}$ percentile.

| | Median µg/L | Range µg/L |
|---|---|---|
| Adults | 0.35 | 0.15 – 0.5 µg/L |

## 8 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or IBL-America directly.


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# 9 PERFORMANCE CHARACTERISTICS

## 9.1 Assay Dynamic Range

The range of the assay is between 0.02 – 10.0 µg/L.

## 9.2 Specificity of Antibodies (Cross Reactivity)

Data can be obtained on request.

## 9.3 Sensitivity

The minimum detectable concentration of C5a by this assay is estimated to be < 0.02 µg/L

## 9.4 Reproducibility

### 9.4.1 Intra Assay

In the range between 2 and 3 µg/L the coefficient of variation in the series (intra-assay CV) was found between 5 and 8%.

### 9.4.2 Inter Assay

In the range between 2 and 3 µg/L the coefficient of variation from day to day (inter-assay CV) was found between 6 and 10%.

## 9.5 Recovery

The recovery of C5a in plasma was between 86 and 114%.

## 9.6 Linearity

Data can be obtained on request.

# 10 LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.

Interferences caused by improper sample handling are explained in the chapters ‘Sample - Collection’.

**Note: Incorrect collection technique, e.g. inadequate mixing of the sample and anti-coagulant, can lead to falsely elevated C5a values!**

## 10.1 Interfering Substances

Haemolytic and lipaemic plasma and plasma containing rheumatoid factors do not interfere with the assay.

## 10.2 High-Dose-Hook Effect

A high-dose hook effect was not observed.


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# 11 LEGAL ASPECTS

## 11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact IBL-America.

## 11.2 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

**Manufactured for :**

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## SYMBOLS USED WITH IBL-AMERICA ELISAS

| Symbol | English | Deutsch | Français | Español | Italiano |
|---|---|---|---|---|---|
| CE | European Conformity | CE-Konfirmitäts-kennzeichnung | Conforme aux normes européennes | Conformidad europea | Conformità europea |
|  | Consult instructions for use | Gebrauchsanweisung beachten | Consulter les instructions d'utilisation | Consulte las Instrucciones | Consultare le istruzioni per l'uso |
| IVD | In vitro diagnostic device | In-vitro-Diagnostikum | Usage Diagnostic in vitro | Para uso Diagnóstico in vitro | Per uso Diagnostica in vitro |
| RUO | For research use only | Nur für Forschungszwecke | Seulement dans le cadre de recherches | Sólo para uso en investigación | Solo a scopo di ricerca |
| REF | Catalogue number | Katalog-Nr. | Référence | Número de catálogo | No. di Cat. |
| LOT | Lot. No. / Batch code | Chargen-Nr. | No. de lot | Número de lote | Lotto no |
|  | 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 | Température de conservation | Temperatura de conservacion | 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 | Vertreiber | Distributeur | Distribuidor | Distributore |
| *Content* | Content | Inhalt | Contenu | Contenido | Contenuto |
| *Volume/No.* | Volume / No. | Volumen/Anzahl | Volume/Numéro | Volumen/Número | Volume/Quantità |
| *Microtiterwells* | Microtiterwells | Mikrotiterwells | Plaques de micro-titration | Placas multipocillo | Micropozzetti |
| *Antiserum* | Antiserum | Antiserum | Antisérum | Antisuero | Antisiero |
| *Enzyme Conjugate* | Enzyme Conjugate | Enzymkonjugat | Conjugué enzymatique | Conjugado enzimático | Tracciante enzimatico |
| *Enzyme Complex* | Enzyme Complex | Enzymkomplex | Complexe enzymatique | Complex enzimático | Complesso enzimatico |
| *Substrate Solution* | Substrate Solution | Substratlösung | Solution substrat | Solución de sustrato | Soluzione di substrato |
| *Stop Solution* | Stop Solution | Stopplösung | Solution d'arrêt | Solución de parada | Soluzione d´ arresto |
| *Zero Standard* | Zero Standard | Nullstandard | Zero Standard | Estándar cero | Standard zero |
| *Standard* | Standard | Standard | Standard | Estándar | Standard |
| *Control* | Control | Kontrolle | Contrôle | Control | Controllo |
| *Assay Buffer* | Assay Buffer | Assaypuffer | Tampon d'essai | Tampón de ensayo | Tampone del test |
| *Wash Solution* | Wash Solution | Waschlösung | Solution de lavage | Solución de lavado | Soluzione di lavaggio |
| *1N NaOH* | 1N NaOH | 1N NaOH | 1N NaOH | 1N NaOH | 1N NaOH (idrossido di sodio 1N) |
| *1 N HCl* | 1 N HCl | 1 N HCl | 1N HCl | 1 N HCl | |
| *Sample Diluent* | Sample Diluent | Probenverdünnungs-medium | Solution pour dilution de l'échantillon | Solución para dilución de la muestra | Diluente dei campioni |
| *Conjugate Diluent* | Conjugate Diluent | Konjugatverdünnungs-medium | Solution pour dilution du conjugué | Solución para dilución del conjugado | Diluente del tracciante |


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