Instructions For Use

eZYDIAG® ELISA RICINE

Quantitative and qualitative assay of ricin toxin

LABORATOIRE AGUETTANT
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France

1 INTENDED USE OF DEVICE

The kit is an in-vitro diagnostic medical device, intended for the specific detection of ricin in human plasma samples. The assay is quantitative and qualitative: a different protocol is defined accordingly.

The kit is intended for any patients, irrespective of their age and sex, potentially exposed and/or poisoned, accidentally or intentionally (suicide, bioterrorism) to the toxic agent Ricin, irrespective of the exposure route (by ingestion, inhalation, or injection).

The tests should be performed on human plasma obtained from blood samples collected in citrate or EDTA collection tubes. Heparinised plasma should not be analysed with the kit.

The kit is intended for use by laboratory professionals, in reference health institutions, and laboratory in designated hospital centres.

The results of the analysis are intended for use by healthcare professionals.

2 PRINCIPLE OF THE ASSAY

The kit is an enzymatic immunoassay (EIA), that uses two ricin-specific monoclonal antibodies (“sandwich” assay) in human plasma samples.

Ricin is a glycoprotein toxin found in the seeds of castor bean plant. This protein has a molecular mass of 66,000 Daltons, and consists of 2 polypeptide chains, A and B, of the same size and connected by a disulphide bridge. Once it is attached to the cell wall via the B chain, the A chain, responsible for the toxic properties, inhibits protein synthesis, resulting in cell death.

A murine capture monoclonal antibody, RB37, targeted against ricin chain B is precoated in the wells of the plate. Samples or standards are then added into the wells. If ricin is present in the samples, it binds to the immobilised antibody (capture). The sandwich is formed by the addition of the second murine monoclonal antibody (Tracer), RA35, targeted against ricin chain A. The enzymatic conjugate is added, and a substrate solution reacts with the enzyme-antibody-target complex to produce a measurable signal. The intensity of this signal is directly proportional to the concentration of target present in the original sample.

The kit may be used according to two protocols:

- A so-called qualitative test protocol, that allows analysing a maximum of samples (up to 45 plasma samples in duplicate), the results are only given on a positive/negative criterion.
- A so-called quantitative test protocol, using a calibration curve and which allows the analysis of a maximum of 38 plasma samples (in duplicate), the results are then given quantitatively (ricin concentration present in the sample).

The solid phase is composed of 12 polystyrene 8-well strip plates, the walls of the wells are coated with the first monoclonal antibody (capture Ab). The second monoclonal antibody (Tracer Ab) is labelled with biotin. The binding of this second antibody is revealed by adding streptavidin conjugated to horseradish peroxidase (HRP).

The number of strip plates to be used should be defined according to the number of samples to be analysed and the protocol chosen using the plate plan proposed (see §9.3 and §10.3).

The test includes the following reaction steps:

1. Pre-washing of the wells to be used (number of strip plates depending on the number of samples to be analysed)
2. Distribution of negative control, positive control (qualitative protocol) or standards range (quantitative protocol) and samples
3. Incubation while stirring
4. Washing followed by distribution of tracer antibody
5. Incubation while stirring
6. Washes, followed by distribution of conjugate
7. Incubation while stirring
8. Washing followed by the revelation of the enzymatic activity linked to the solid phase by addition of the substrate
9. Stopping revelation
10. Spectrophotometer reading of the plate, absorbance at 450 nm and 630 nm (or 620 nm depending on the filters available)
11. Interpretation of results

Performing the test in a room at a temperature above 25°C may cause a deterioration of the performance.

3 KIT COMPOSITION

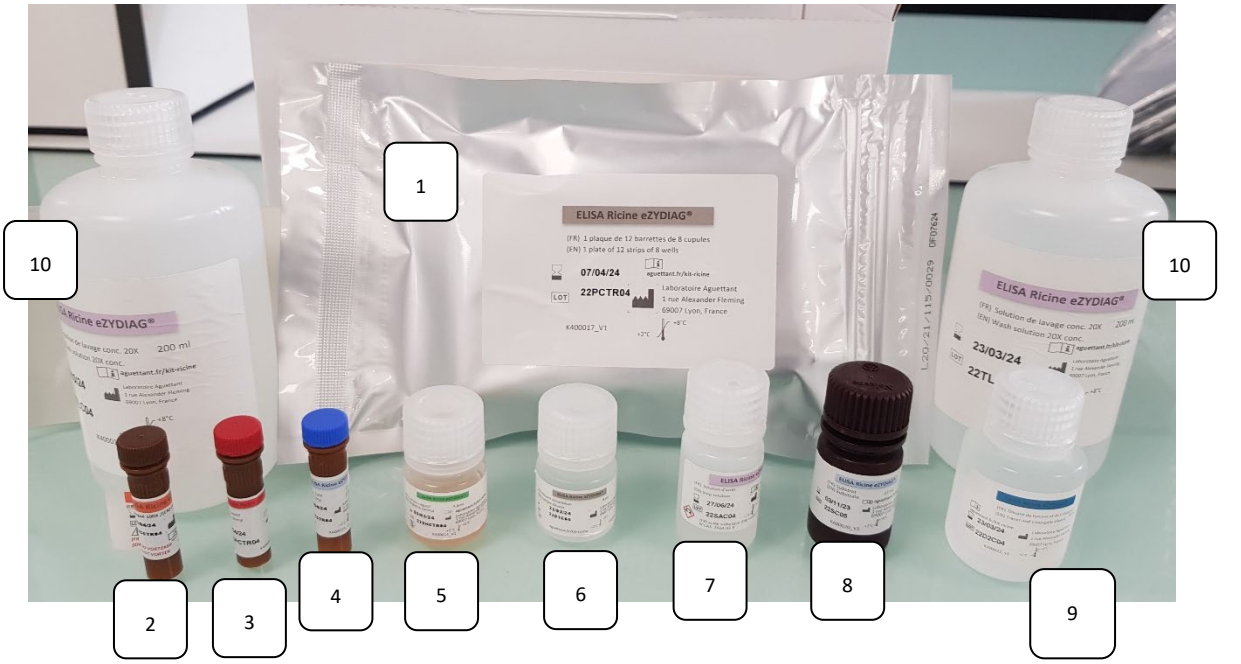

Figure 1: Kit composition

Components	Nature of reagents	Presentation	No.
RICIN ELISA PLATE	Microplate: 12 8-well strip plates coated with anti-ricin mouse monoclonal antibody	1 plate with desiccant in resealable aluminium-coated sachet	1
CONJUGATE 100X	Streptavidin conjugated to horseradish peroxidase (HRP) solution. **100 times concentrate solution (100X)**	1 vial (0.2 mL)	2
POSITIVE CONTROL	Positive control. **To be diluted according to the protocol to obtain the positive control (qualitative protocol) or the calibration range (quantitative protocol).**	1 vial (1.1 mL)	3
TRACER 100X	Solution of detection antibodies (mice anti-ricin monoclonal antibodies) conjugated to biotin. **100 times concentrate solution (100X)**	1 vial (0.2 mL)	4
CONTROL -	Negative control composed of controlled foetal calf serum. **To be diluted according to the protocol.**	1 vial (4.8 mL)	5
SAMPLE DILUENT	Dilution buffered solution (phosphate saline), contains a non-specific mice monoclonal antibody. **Ready-to-use solution**	1 vial (8 mL)	6
STOP SOLUTION	Stop solution, 2.95 % sulphuric acid **Ready-to-use solution** 	1 vial (12 mL)	7
SUBSTRATE	TMB solution (3,3´, 5,5´-tetramethylbenzidine). **Ready-to-use solution**	1 vial (13 mL)	8
TRACER AND CONJUGATE DILUENT	Tracer and conjugate dilution buffered solution (phosphate buffer saline). **Ready-to-use solution**	1 vial (28 mL)	9
WASH SOLUTION 20X	Buffered solution **20 times concentrated solution (20X).**	2 bottles (2x200 mL)	10
/	Adhesive films	9 adhesive films	11

Metrological traceability: POSITIVE CONTROL is prepared from ricin meal. Value assigned is controlled according to a calibration hierarchy defined internally.

4 MATERIALS REQUIRED BUT NOT PROVIDED

The use of the kit requires using the following materials, not supplied by LABORATOIRE AGUETTANT:

- **Micropipettes**. This equipment must be in good condition and undergo regular metrological verifications in accordance with good laboratory practice.
- **8-Channel micropipette**. This apparatus must be in good condition and undergo regular metrological verifications in accordance with good laboratory practice.
- **Polypropylene tubes** to perform the different dilutions.
- **Microplate stirrer**
- **Automatic plate washer** (manual washing does not meet the validation criteria of the results). This apparatus must be in good condition and undergo regular metrological verifications in accordance with good laboratory practice.
- **Spectrophotometer** that can measure absorbance at 450 nm and 630 nm (or 620 nm depending on the filters available) on 96-well microplates. This instrument must be in good condition and undergo regular metrological verifications in accordance with good laboratory practice.

IT IS ESSENTIAL TO HAVE A MICROPLATE WASHER

IT IS STRONGLY ADVISE AGAINST TO PERFORM THE WASHING BY INVERTING

2 washing programmes are used: (to be programmed on your washer)

- **Programme no. 1 (Prog 1):** 3 successive distributions of 300 µL per well **with** final aspiration.
- **Programme no. 2 (Prog 2):** 3 successive distributions of 300 µL per well **without** final aspiration.

It is essential to respect these washing protocols for the quality and validity of the results.

5 STABILITY AND STORAGE CONDITIONS

- Store the kit in its original packaging, at a temperature ranging between **+2°C and +8°C**.
- Do not use the kit after the expiry date indicated on the kit label.
- Do not freeze the reagents.
- The kit can be reused within 1 month after opening in the following conditions:
    - Rapidly after use, components are stored between +2°C and +8°C.
    - Liquid reagents are not diluted or soiled
    - Bottles, vials and sachet are carefully closed after use.
    - The RICIN ELISA PLATE sachet was opened only after return to room temperature.
    - It is recommended to record the date of first use.

6 TYPES OF SAMPLES

The test should be performed on human plasma obtained from blood samples collected in citrate or EDTA collection tubes. Heparinised plasma should not be analysed with this kit.
It is preferable to test the samples within 24 hours following the collection with storage between +2°C and +8°C. Beyond that, the storage of plasma is possible at -20°C for a duration of less than 6 months, or at -70/80°C for longer periods.
Follow the standard blood collection procedure avoiding any direct contact with the blood.
Since some samples could generate false results, it is recommended to identify all the samples which may present an abnormal appearance (opalescent, lactescent, icteric, haemolysed, partially coagulated, etc.) prior to analysis.

If the samples need to be transported, they must be packed according to the regulations concerning the transport of biological samples and transported refrigerated (between +2°C and +8°C) for a maximum duration of 25 hours or frozen (< -20°C).

7 WARNINGS AND PRECAUTIONS

- Do not use the kit if damaged.
- Do not use the kit if there are signs of contamination or modification.
- The reagents must be stored at a temperature between +2°C and +8 °C.
- Do not use reagents after the expiry date.
- Do not mix or combine, during the same experiment, reagents from kits from different batches.
- Prepare the samples immediately before use.
- Performing the test in a room at a temperature above 25°C may cause a deterioration of the performance of the kit.
- Allow the reagents to come to room temperature over approximately one hour (except TRACER 100X and CONJUGATE 100X) before use.
- Do not vortex the TRACER 100X and CONJUGATE 100X as well as their dilution (mix by aspiration – discharge).
- Carefully dilute the reagents, avoiding contamination.
- STOP SOLUTION contains sulfuric acid and represents a corrosive hazard, so please take precautions when using it.
- Do not use glassware for preparing the reagents, favour disposable containers.
- It is not recommended to pipet very small volumes (<10 µL).
- After each wash, the reagents must be distributed as quickly as possible without delay.
- Change the pipette tip between each sample.
- Follow strictly the protocol provided by the manufacturer.
- Washing the wells is an essential step of the procedure and must be performed with a microplate washer, washing by inverting is strongly discouraged.

- Respect the washing programmes indicated in these instructions for use and verify that all the wells are completely filled, then totally emptied. A poorly performed wash will result in incorrect results.
- Never use the same container and the same pipette tip to add the conjugate and the substrate.
- On partially coagulated samples, make sure not to pipet fibrin.
- In a qualitative protocol context, systematically test the positive and negative controls.
- In a quantitative protocol context, systematically integrate a calibration range to all experiments.
- In case of identification of strongly concentrated positive samples, it is recommended to inactivate the liquid waste with sodium hydroxide (0.1 M NaOH at final concentration).
- Verify that all the apparatuses used in this procedure have been verified and calibrated according to the manufacturer's recommendations.
- Handle carefully and safely kit components: the CONTROL - and sample diluent include substances from animal origin (risk of infection) and positive control present a biological hazard.

(*) The material safety data sheets of the kit are provided by LABORATOIRE AGUETTANT to the privileged client interlocutor with any order of the kit. They are available at https://www.aguettant.fr/kit-ricine/.

8 PREPARATION OF REAGENTS

The entire preparation of reagents and dilution of samples should be performed immediately before use.

8.1 Ready-to-use reagents

8.1.1 RICIN ELISA PLATE

Before opening the sachet, allow the RICIN ELISA PLATE to return to room temperature for 1 hour in its protective packaging, in order to prevent any condensation in the wells.
Right before depositing the samples, open the sachet to the seal point, take the number of plate strips required for the analyses and place the unused strip plates immediately in the sachet with the desiccant. Close tightly after carefully removing the air.
Keep at a temperature between +2°C and +8 °C.

8.1.2 Ready-to-use liquid reagents

The following reagents:

- TRACER AND CONJUGATE DILUENT
- SAMPLE DILUENT
- SUBSTRATE
- STOP SOLUTION
- CONTROL -
- POSITIVE CONTROL

must be returned to room temperature, approximately 1 hour before their use.

Leave the TRACER 100X and CONJUGATE 100X between +2°C and +8 °C.
After each use, they must be closed carefully and placed rapidly between +2°C and +8°C to guarantee a good storage after opening.

8.2 Dilution of reagents

After each use, reagents must be closed carefully and placed rapidly between +2°C and +8 °C to guarantee a good storage. Only use the amount required to perform the analyses.

8.2.1 DILUTED TRACER

The preparation of the DILUTED TRACER should be performed no more than 10 minutes before the end of the incubation of samples and standards.
Dilute the TRACER 100X to 1/100 in TRACER AND CONJUGATE DILUENT (e.g.: 10 µL of TRACER 100X + 990 µL of TRACER AND CONJUGATE DILUENT for one strip plate). Perform a gentle aspiration-discharge stirring with a micropipette or by inversion to homogenise the mixture. Do not vortex.
It is recommended to avoid pipetting very small amounts (< 10 µL). As a reminder, micropipettes in good condition and which have recently been calibrated must be used.

8.2.2 DILUTED CONJUGATE

The preparation of the DILUTED CONJUGATE should be performed 10 minutes before depositing in the well.

Dilute the CONJUGATE 100X to 1/100 in TRACER AND CONJUGATE DILUENT (e.g.: 10 µL of CONJUGATE 100X + 990 µL of TRACER AND CONJUGATE DILUENT). Perform a gentle aspiration-discharge stirring with a micropipette or by inversion to homogenise the mixture. Do not vortex.
It is recommended to avoid pipetting very small amounts (< 10 µL). As a reminder, micropipettes in good condition and which have recently been calibrated must be used.

8.2.3 DILUTED WASH SOLUTION

Dilute the WASH SOLUTION 20X to 1/20 in distilled water, ultrapure water or “Water for injections” at room temperature (e.g.: 50 mL of WASH SOLUTION 20X + 950 mL of distilled water).

9 QUALITATIVE TEST PROTOCOL

9.1 Preparation of controls for the qualitative protocol

Identification of the point	Dilution to be made	Reagent 1	Volume Reagent 1 (µL)	Reagent 2	Volume Reagent 2 (µL)
Positive Control	¼	POSITIVE CONTROL	60	SAMPLE DILUENT	180
Negative Control	½	CONTROL -	250	SAMPLE DILUENT	250

9.2 Preparation of the samples

The samples must be tested in duplicate.

Identification of the point	Dilution to be made	Reagent 1	Volume Reagent 1 (µL)	Reagent 2	Volume Reagent 2 (µL)
Sample x	½	Sample x	125	SAMPLE DILUENT	125

9.3 Plate plan (qualitative)

The plate diagram proposed below should be respected in order to allow a simplified use of the Excel file for result processing.

This file is provided by LABORATOIRE AGUETTANT to the privileged client interlocutor with any order of the kit and is available at https://www.aguettant.fr/kit-ricine/.

	Enter the identifier of each of your samples in the boxes											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	*Positive Control*	SAMP no.2	SAMP no.6	SAMP no.10	SAMP no.14	SAMP no.18	SAMP no. 22	SAMP no. 26	SAMP no.30	SAMP no. 34	SAMP no. 38	SAMP no. 42
B												
C	*Negative Control*	SAMP no.3	SAMP no.7	SAMP no.11	SAMP no.15	SAMP no.19	SAMP no. 23	SAMP no. 27	SAMP no.31	SAMP no. 35	SAMP no. 39	SAMP no. 43
D												
E		SAMP no. 4	SAMP no. 8	SAMP no. 12	SAMP no. 16	SAMP no. 20	SAMP no. 24	SAMP no. 28	SAMP no. 32	SAMP no. 36	SAMP no. 40	SAMP no. 44
F												
G	SAMP no. 1	SAMP no. 5	SAMP no. 9	SAMP no. 13	SAMP no. 17	SAMP no. 21	SAMP no. 25	SAMP no. 29	SAMP no. 33	SAMP no. 37	SAMP no. 41	SAMP no. 45
H												

(SAMP = sample)

9.4 Detailed qualitative protocol

Before any incubation, cover the plate with a clean adhesive film (included in the kit).

1. Wash the strip plates before use with 3 successive distributions of 300 µL of DILUTED WASH SOLUTION (see §8.2.3) per well (programme No. 1, see §4).
2. According to the plate diagram, distribute 100 µL of *diluted positive control* (see §9.1) in duplicate and 100 µL of the *diluted Negative control* (see §9.1) in quadruplicate as well as 100 µL of each *diluted sample* to be analysed (see §9.2).
3. Incubation while stirring (1000 rpm) for 45 minutes at room temperature.
4. Wash with 3 successive distributions of 300 µL of DILUTED WASH SOLUTION (see §8.2.3) per well (programme no. 1, see §4).
5. Distribute 100 µL of DILUTED TRACER (see §8.2.1) per well.
6. Incubation while stirring (1000 rpm) for 45 minutes at room temperature.
7. Wash with 3 successive distributions of 300 µL of DILUTED WASH SOLUTION (see §8.2.3) per well (programme No. 1, see §4).
8. Distribute 100 µL of DILUTED CONJUGATE (see § 8.2.2) per well.
9. Incubation while stirring (1000 rpm) for 30 minutes at room temperature.
10. Wash with 3 successive distributions of 300 µL of DILUTED WASH SOLUTION (see §8.2.3) per well **without final aspiration** (programme No. 2, see §4).
11. Stir 5 minutes at 1000 rpm wells containing the 300 µL of DILUTED WASH SOLUTION (see §8.2.3).
12. Wash with 3 successive distributions of 300 µL of DILUTED WASH SOLUTION (see §8.2.3) per well (programme No. 1, see §4).
13. Distribute 100 µL per well of SUBSTRATE.
14. Incubate in the dark for 30 minutes without stirring.
15. Distribute 100 µL per well of STOP SOLUTION.
16. Read the absorbance at 450 and 630 nm (or 620 nm depending on the filters available).

If applicable, program the reader to obtain directly the values corresponding to the subtraction of the absorbances measured at 450 nm from those measured at 630 nm. Otherwise, this subtraction should be carried out on a spreadsheet or manually, from the raw data of the reader.

9.5 Calculation and interpretation of results (qualitative protocol)

The interpretation of the results of the qualitative protocol is facilitated by the use of an Excel file designed for this purpose. This file is provided by LABORATOIRE AGUETTANT to the privileged client interlocutor with any order of the kit. The file is available at https://www.aguettant.fr/kit-ricine/

The file has 3 sheets, “General information”, “Plate diagram - Entry of readings” and “Results”

9.5.1 “General information” Sheet

This sheet allows to freely enter the information you desire.

9.5.2 "Plate diagram - Entry of readings" Sheet

This sheet allows you to enter the name of your laboratory, the date, identity of the operator, the reference of the kit used.

It informs you about the plate diagram to be followed ("Plate diagram" table).

The boxes "Samp No.X" ("Plate diagram" table) are accessible to enter the identifier of each of your samples.

The table in the bottom allows you to enter manually or by "copy-paste" all the experimental data (OD450nm minus OD630nm) of each well **according to the plate diagram** and the number of samples tested.

When entering/copying the data, the number format (in particular the decimal separator "," or ".") according to the parameters of your operating system and/or Excel.

Warning, in case of number format error during entry or copy (wrong decimal separator), no calculation can be performed. In that case, the text "Entry pb" will be displayed in red above the column on the result entry table (error test performed only online "A" of the table).

You can invalidate the values that seem aberrant to you (to be defined under your responsibility), both for controls and samples. To do this, the value in question has to be deleted in the entry of results table.

A "Comments/Observations" field allows indicating any additional information you consider useful.

9.5.3 "Results" Sheet

On this sheet, the only fields accessible for entry by the user are the "Operator initials" and "Validation" cells.

This calculation and results report page is automatically filled in from the data entered in the "Plate diagram - Entry of readings" tab.

In the event that no calculation appears on the "Results" sheet, verify that you actually used "." as the decimal separator when entering the data.

The acceptance criteria for the controls are the following:

Negative control	OD (450-630nm) ≤ **0.050**
Positive control	OD (450-630 nm) ≥ **0.700**

After validation of these criteria, the following ratio is calculated for each sample:

$$\frac{[Mean\ (OD450nm\ -\ OD630\ nm\ Sample)]}{[Mean\ (OD450nm\ -\ OD630\ nm\ negative\ control)]}$$

If this ratio is **less than or equal to 2.00**, the sample is **negative**.

If this ratio is **greater than 2.00** the sample is **potentially positive** and must be re-tested using the **quantitative protocol**.

In case of non-validation of one or both controls, the results of the samples cannot be considered and validated (text "**Invalid result**" in the result sheet). The assay needs then to be repeated, after verifying at least the following elements:

- Verify the absence of an aberrant point among the 4 points of Standard 0.
- General compliance with the recommendations and protocols of this leaflet.
- The preparation of reagents in compliance with protocol provided.
- Programming of plate washer used.
- Proper function of the washer.
- Compliance with pre-washing of strip plates right at the beginning of the protocol.
- The use of 2 washing programmes according to the protocol provided.
- Room temperature.
- The wavelengths used during the readings.

10 QUANTITATIVE TEST PROTOCOL

10.1 Preparation of calibration curve

Volumes are calculated considering a test of standards in duplicate.

Identification of the point	Dilution to be made	Reagent 1	Volume Reagent 1 (µL)	Reagent 2	Volume Reagent 2 (µL)
Standard 0	½	CONTROL -	1300	SAMPLE DILUENT	1300
Standard 500 pg/mL	¼	POSITIVE CONTROL	120	*Standard 0*	360
Standard 250 pg/mL	½	*Standard 500*	240	*Standard 0*	240
Standard 125 pg/mL	½	*Standard 250*	240	*Standard 0*	240
Standard 62.5 pg/mL	½	*Standard 125*	240	*Standard 0*	240
Standard 31.2 pg/mL	½	*Standard 62.5*	240	*Standard 0*	240
Standard 15.6 pg/mL	½	*Standard 31.2*	240	*Standard 0*	240

10.2 Preparation of the samples

The samples must be tested in duplicate.

Identification of the point	Dilution to be made	Reagent 1	Volume Reagent 1 (µL)	Reagent 2	Volume Reagent 2 (µL)
Sample x	½	Sample x	125	SAMPLE DILUENT	125

10.3 Plate diagram (quantitative)

The plate diagram proposed below should be respected in order to allow a simplified use of the Excel file for result processing. This file is provided by LABORATOIRE AGUETTANT to the privileged client interlocutor with any order of the kit. The file is available at https://www.aguettant.fr/kit-ricine/

	Standards			Enter the identifier of each of your samples in the boxes								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	STD 500	STD 31.25	STD 0	SAMP no.3	SAMP no.7	SAMP no.11	SAMP no.15	SAMP no.19	SAMP no. 23	SAMP no. 27	SAMP no.31	SAMP no. 35
B												
C	STD 250	STD 15.6		SAMP no. 4	SAMP no. 8	SAMP no. 12	SAMP no. 16	SAMP no. 20	SAMP no. 24	SAMP no. 28	SAMP no. 32	SAMP no. 36
D												
E	STD 125	STD 0	SAMP no. 1	SAMP no. 5	SAMP no. 9	SAMP no. 13	SAMP no. 17	SAMP no. 21	SAMP no. 25	SAMP no. 29	SAMP no. 33	SAMP no. 37
F												
G	STD 62.5		SAMP no.2	SAMP no.6	SAMP no.10	SAMP no.14	SAMP no.18	SAMP no. 22	SAMP no. 26	SAMP no.30	SAMP no. 34	SAMP no. 38
H												

(STD: Standard / SAMP: Sample)

10.4 Detailed quantitative protocol

Before any incubation, cover the plate with a clean adhesive film (included in the kit).

1. Wash the number of strip plates required before use with 3 successive distributions of 300 µL of DILUTED WASH SOLUTION (see §8.2.3) per well (programme No. 1, see §4).
2. According to the plate diagram, distribute in duplicate, 100 µL of *standards 500 pg/mL to 15.6 pg/mL* (see §10.1) and distribute 100 µL of *standard 0* (see §10.1) in the 8 wells indicated and distribute in duplicate 100 µL of each *diluted sample* to be analysed (see §10.2).
3. Incubate while stirring (1000 rpm) for 45 minutes at room temperature.
4. Wash with 3 successive distributions of 300 µL of DILUTED WASH SOLUTION (see §8.2.3) per well (programme No. 1, see §4).
5. Distribute 100 µL of DILUTED TRACER per well (see §8.2.1).
6. Incubate while stirring (1000 rpm) for 45 minutes at room temperature.
7. Wash with 3 successive distributions of 300 µL of DILUTED WASH SOLUTION (see §8.2.3) per well (programme No. 1, see §4).
8. Distribute 100 µL of DILUTED CONJUGATE per well (see §8.2.2).
9. Incubate while stirring (1000 rpm) for 30 minutes at room temperature.
10. Wash with 3 successive distributions of 300 µL of DILUTED WASH SOLUTION (see §8.2.3) per well **without final aspiration** (programme No. 2, see §4).

11. Stir 5 minutes at 1000 rpm (wells containing the 300 µL of DILUTED WASH SOLUTION).
12. Wash with 3 successive distributions of 300 µL of DILUTED WASH SOLUTION (see §8.2.3) per well (programme No. 1, see §4).
13. Distribute 100 µL of SUBSTRATE per well.
14. Incubate in the dark for 30 minutes without stirring.
15. Distribute 100 µL of STOP SOLUTION per well.
16. Read the absorbance at **450 and 630 nm** (or 620 nm depending on the filters available). If you can, programme your reader to obtain directly the values corresponding to the subtraction of the absorbances measured at 450 from those measured at 630 nm. Otherwise, this subtraction should be carried out on a spreadsheet or manually, from the raw data of the reader.

10.5 Interpretation of results (quantitative protocol)

The interpretation of the results of the quantitative protocol is facilitated by the use of an Excel file designed for this purpose.

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The file has 3 sheets, “General information”, “Plate diagram - Entry of readings” and “Results”

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This sheet allows you to enter the name of your laboratory, the date, identify of the operator, the references of the kit used.

It informs you about the plate diagram to be followed (“Plate diagram” table).

The boxes “Samp No.X” (“Plate diagram” table) are accessible to enter the identifier of each of your samples.

The table in the bottom allows you to enter manually or by “copy-paste” all the experimental data (OD450nm minus OD630nm) of each well **according to the plate diagram** and the number of samples tested.

When entering/copying the data, the number format (in particular the decimal separator “,” or “.”) according to the parameters of your operating system and/or Excel.

Warning, in case of number format error during entry or copy (wrong decimal separator), no calculation can be performed. In that case, the text “Entry pb” will be displayed in red above the column on the result entry table (error test performed only on line “A” of the table).

You can invalidate the values that seem aberrant to you (to be defined under your responsibility), both for controls and samples. To do this, the value in question has to be deleted in the entry of results table.

A “Comments/Observations” field allows you to indicate any additional information you consider useful.

10.5.3 "Results" Sheet

On this sheet, the only fields accessible for entry by the user are the "Operator initials" and "Validation" cells (to be filled in according to your procedures).

This calculation and results report page is automatically filled in from the data entered in the "Plate diagram - Entry of readings" tab.

In the event that no calculation appears on the "Results" sheet, verify that you actually used "." as the decimal separator when entering the data.

The acceptance criteria for the standard range are the following:

Standard 0	Mean OD (450-630nm) Std 0	≤ **0.050**
Standard 500	Mean OD (450-630 nm) Std 500	≥ **0.700**
Limit of detection < Limit of quantification		

The concentration of the samples is calculated after validation of these criteria.

- Any sample with a result below the Limit of Detection (LOD) is considered as **negative**.
- Any sample for which the result is comprised between the limit of detection (LOD) and the limit of quantification (LOQ) is considered as "**non quantifiable positive**". It is recommended to re-test it for verification.
- Any sample for which the result is greater than the limit of quantification (LOQ) is considered as **positive,** and the concentration displayed in the result table shall be multiplied by 2 to have the final concentration in the patient sample.


Results displayed in the Excel file are concentrations per well. Thus, the sample being diluted 1:2 during preparation, the result obtained should be **multiplied by 2** to get the real concentration in the patient sample.

The text "**Invalid result**" in the result sheet indicates that the acceptance criteria of the standard range have not been attained. The source of these abnormalities could be essentially related to:

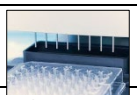
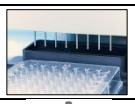
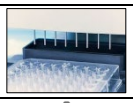
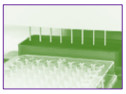
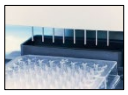
- Abnormal results in Standard 500.
  - Verify the absence of an aberrant point in the duplicate of Standard 500.
  - Delete the aberrant point from the entry table.
  - Verify that the dilution of standard range protocol was complied with.
- Abnormal results in Standard 0.
  - Verify the compliance with pre-washing of strip plates right at the beginning of the protocol.
  - Then, verify the absence of an aberrant point among the replicates of Standard 0.
  - Delete the aberrant points from the entry table (do not delete more than 2 points).
  - In case of major lack of reproducibility (more than 2 aberrant points), it is recommended to repeat the test with the utmost rigour, especially for the washing.

- A very high general signal level of all Standards 0.
    - Verify the programming of the plate washer used.
    - Verify the proper function of this washer.
    - Verify the compliance with pre-washing of strip plates right at the beginning of the protocol.
    - Verify the use of 2 washing programmes according to the protocol provided.
    - Verify the room temperature.
    - Verify the stirring parameters.

The three sheets of the Excel file can be printed.

In the even that no calculation appears on the “Results” sheet, verify that you actually used “.” as the decimal separator.

11 SCHEMATIC GENERAL PROTOCOL

1 – Wash the RICIN ELISA PLATE immediately before depositing the samples	3 times 300 µL/well (Prog 1)	
2 – Deposit the controls or the standards (depending on the protocol chosen) and deposit the diluted samples	100 µL/well	
3 - Incubate while stirring (1000 rpm)	**45 minutes** Room temperature	
4 – Wash the plates	3 times 300 µL/well (Prog 1)	
5 - Deposit the DILUTED TRACER	100 µL/well of TRACER 100X diluted to 1/100	
6 - Incubate while stirring (1000 rpm)	**45 minutes** Room temperature	
7 – Wash the plates	3 times 300 µL/well (Prog 1)	
8 - Deposit the DILUTED CONJUGATE	100 µL/well of CONJUGATE 100X diluted to 1/100	
9 - Incubate while stirring (1000 rpm)	**30 minutes** Room temperature	
10 - Wash the plates (Prog 2)	3 times 300 µL/well **without final aspiration (Prog 2)**	
11 - Incubate	**5 minutes** at room temperature while stirring (without adhesive film) (1000 rpm)	
12 – Wash the plates	3 times 300 µL/well (Prog 1)	
13 - Deposit the SUBSTRATE	100µL/ well	
14 - Incubate	**30 minutes** Room temperature - protected from light	
15 – Deposit the STOP SOLUTION	100 µL/well	
16 - Read in bi-chromatic mode	450/630 nm	
17 – Subtract the signals of the 2 wavelengths	[OD 450 nm - OD 630 nm]	
18 - Interpret	Qualitative: see §9.5 Quantitative: see §10.5	

12 HEALTH AND SAFETY INSTRUCTIONS

In general: the health, safety and good laboratory practice conditions must comply with the various applicable regulations.

- All the reagents of the kit are intended exclusively for *in vitro* diagnosis.
- Wear disposable gloves while handling the reagents and samples and wash hands thoroughly after handling.
- Never mouth pipet.
- Use the personal protective equipment recommended in the procedures of your laboratory.

The waste derived from the use of the kit should be discarded as biological and chemical waste.

13 PERFORMANCE CHARACTERISTICS

13.1 Performance

Repeatability	Optical Density Standard deviation ≤ 20 %
Intermediate precision	Optical Density Coefficient of variation ≤ 25 %
Analytical sensitivity (Limit of detection)	15.25 pg/mL Negative specimens: DO ≤ 0,050 Positive specimens: DO ≥ 0,700 Positivity threshold ratio [average(OD 450nm- OD 630nm sample)] / [average(OD 450nm- OD 630nm negative control)] > 2.
Measuring range (quantitative assay)	15.625 pg/mL - 500 pg/mL
Analytical specificity	> 95 %
Clinical performance	Only analytical performance studies on spiked samples were performed.

13.2 Interfering substances and limitations

Some samples containing high concentrations of rheumatoid factor (RF) could result in non-specific positive results. Such cases must be identified before performing the test.

The test has been designed to eliminate any potential interference with human anti-mouse antibodies (HAMA). However, high concentrations of HAMA may result in false positive results.

As is the case for any diagnostic procedure, the physician should evaluate the result obtained via this kit with respect to the other clinical and diagnostic information available.

Do not use heparinised samples.

Patients showing questionable results should be tested again using a new sample.

RA35 was selected as Tracer antibody with RB37 as capture antibody for their capacity to recognise ricin with very high sensitivity in sandwich immunoassays. Thus, this immunological assay method is very specific and cannot cross react with other ingredients (medicinal products, endogenous substances, food substances, anticoagulants or preservatives or other similar compounds).

14 ASSISTANCE AND COMPLAINT

For technical assistance, please contact LABORATOIRE AGUETTANT:

- www.aguettant.fr or
- By telephone:(33) 04 78 61 51 41

Medical information: infoscientifique@aguettant.fr

Complaints should be reported to: reclamations@aguettant.fr
Incidents should be reported to: materiovigilanceAGT@aguettant.fr

Any serious incident with the device should be reported to the manufacturer and to the competent authorities of the Member State in which the user and/or patient reside.

15 INDEX OF SYMBOLS

	In vitro diagnosis medical device		CE marking
	Read the instructions For Use Instruction for Use available at the address www.aguettant.fr/kit-ricine		Warning
	Batch number		Biological risks
	Expiry date		Contains animal biological material
	Manufacturer		Do not use if packaging is damaged and consult the Instructions For Use
	Store between +2°C and +8°C		Product reference
	Danger: corrosive (GHS05)		

16 BIBLIOGRAPHY

N/A

17 MODIFICATIONS HISTORY

Version	Date	Summary of modifications
04	07/2024	Harmonisation of components name. Review of performance section. Addition of a warning regarding infection risk and biological hazard. Editorial changes.
03	04/2024	Addition of warning to multiplied by 2 to get the final patient result.
02	08/2023	Shelf-life: removal of 24 months to refer to the expiry date on the kit label instead.
01	10/2022	Creation