biocross

Ref. 3135005

e4Risk® Reagents

CONTENTS
e4Risk® R1. 1 x 10 mL
e4Risk® R2. 1 x 10 mL

Latex turbidimetry
For in vitro diagnostic use only

CE

PRINCIPLE

e4Risk® is a latex agglutination test based on the turbidimetric principle for the quantitative in vitro determination of the isoform E4 of the apolipoprotein E (ApoE4) in human plasma samples. The test consists on one reagent with latex particles and another reagent with a mouse anti-ApoE4, which induces the latex particles agglutination when ApoE4 is present in the plasma sample. This agglutination can be measured with a photometer. The degree of turbidity is proportional to the sample concentration of ApoE4.

REAGENTS COMPOSITION

e4Risk® R1. Ref.: 3035050 1 x 10 mL. anti-apoE4 antibody in a Bis-Tris buffer, BSA ≤0.5 %, NaN3 < 0.1%.

e4Risk® R2. Ref.: 3035056 1 x 10 mL. Latex particles, in a Tris buffer, NaN3 < 0.1%.

e4Risk® Calibrator. Ref.: 3935006 1 x 0.5 mL human recombinant ApoE4 in human plasma. ApoE4 concentration is stated on the vial.

e4Risk® Positive Control. Ref.: 3935011. 1 x 0.5 mL. Pool of Human plasma from *APOE* ε4 carriers and *APOE* ε4 non-carriers.

e4Risk® Negative control. Ref.: 3935012. 1 x 0.5 mL. Pool of Human plasma from *APOE* ε4 carriers and *APOE* ε4 non-carriers.

Precautions: The reagents R1 and R2 contain sodium azide <0.1%. Avoid any contact with skin or mucous membranes.

The calibrator and controls contain human plasma. Material should be treated as potentially infectious.

REAGENT PREPARATION

e4Risk® R1. Ready to use.

e4Risk® R2. Ready to use. Gently mix the vial before use avoiding the formation of foam.

e4Risk® Calibrator. Freeze-dried. Reconstitute with 0.5 mL of purified water.

e4Risk® Positive control. Freeze-dried. Reconstitute with 0.5 mL of purified water.

e4Risk® Negative control. Freeze-dried. Reconstitute with 0.5 mL of purified water.

Calibration curve (2 points): Calibrator curve will be prepared with CAL and purified water: Prepare a tube with purified water (calibrator 0 µg/L). Reconstitute CAL tube just prior to use. Allow CAL tube to reach room temperature for 10 minutes before resuspending with 0.5 mL purified water. Allow 15 minutes for the calibrator material to dissolve then mix gently for 15 minutes on the roller avoiding the creation of bubbles.

STORAGE AND STABILITY

1. Unopened reagents will remain stable until the expiration date printed on the label only when stored tightly closed at 2-8 °C and contaminations are prevented during their use.
2. R1 and R2 will remain stable after opening for at least 2 months when stored tightly closed at 2-8ºC. Do not leave reagents open for a long period. Evaporation may cause concentration of R1 that may impact measurements. Reconstituted calibrator and controls are stable after reconstitution for 1 and 7 days, respectively at 2-8ºC.
3. Do not interchange reagents from different lots. Do not use the reagents after the expiration date.
4. Reagent deterioration: Presence of particles and turbidity.

SAMPLE

e4Risk® is designed to be used in human plasma samples. Other matrices such serum or blood have not been tested. For plasma collection, use standard venipuncture techniques into EDTA tubes. Other anticoagulants have not been tested and might interfere with the results. Centrifuge blood at 2280 rcf for 10 min at 2-8ºC and separate plasma as soon as possible after collection.

Isolated plasma is stable at least for 24 hours if kept at room temperature (20-25ºC) and up to 7 days if stored at 2-8ºC. Plasma samples can be frozen and kept at -80 ± 10ºC for at least 3 months. Result is not changed after 2 freeze/thaw cycles. Frozen plasma samples can be also stored at -20 ± 5ºC for at least 3 months and result is unaltered after 1 freeze/thaw cycle.

MATERIALS REQUIRED

- Thermostable Spectrophotometer or photometer thermostable at 37ºC with a 546 filter.

PROCEDURE

Sample procesing:

If plasma cannot be processed immediately after isolation, keep plasma at 2-8ºC until use.

Preparation of controls:

Reconstitute controls just prior to use. Allow C+ and C- tubes to reach room temperature for 10 minutes before reconstituting with 0.5 mL purified water. Allow 15 minutes for the control materials to dissolve then and mix gently for 15 minutes on the roller avoiding the creation of bubbles.

Analitycal Procedure

1. Zero out the intrument at 546 nm using distilled water
2. Pipette the following components into a cuvette:

R1	550 µL
Sample / Calibrator / Controls / Water (blank)(*)	25 µL
R2	550 µL

(*) Minimum volume 5 µL. Keep volume proportions between sample/calibrator/controls/water vs R1 and R2.

3. Mix well and read the absorbance immediately (A_1).
4. Incubate tmixture at 37ºC for 5 minutes and read the absorbance (A_2)
5. Perform calculations.

Calculation

Calculate the absorbance difference (A_2-A_1) of each point of the calibration curve and plot the values obtained against the ApoE4 concentration of both calibration points. ApoE4 concentration in the sample is calculated by interpolation of its (A_2-A_1) value in the calibration curve.

QUALITY CONTROLS

e4Risk® controls are recommended to monitor the performance of manual and automated assay procedures. It is recommended to use positive and negative Controls (ref: 3935011 and 3935012 respectively).

Each laboratory should establish its own Quality Control scheme and corrective actions should be made if controls do not meet the acceptable tolerances.

EXPECTED VALUES

Biocross recommends a cut-off of 4.62 μg/mL. Although analysis is quantitative (measured in μg/mL), results should be reported qualitatively (*APOE ε*4 carrier / *APOE ε*4 non-carrier). Therefore, samples with a concentration above the cut-off are considered to come from *APOE ε*4 carriers (patients with at least one ε4 allele of the *APOE* gene), while samples with a concentration below the cut-off are considered to come from *APOE ε*4 non-carriers (patients with no copies of ε4 allele of the *APOE* gene),

CLINICAL SIGNIFICANCE

e4Risk® is an immunoturbidimetric assay for the evaluation of the *APOE ε*4 carriership in human plasma samples.

To date, only the presence of one or two alleles ε4 of the *APOE* gene is accepted as a reliable biomarker and risk factor of developing late onset Alzheimer´s Disease (AD)[1, 2]. *APOE* ε4 is present in approximately 20% of the global population and in 40-60% of all patients with late-onset AD[3, 4]. The presence of one allele ε4 of the *APOE* gene increases the risk of suffering AD by 3-5 fold, while the presence in homozygosis increases the risk by 15-20 fold[5]. Furthermore, AD manifests earlier and progresses more rapidly in *APOE* ε4 carriers than in *APOE* ε4 non carriers[6, 7].

e4Risk® will inform the clinician regarding the patient´s risk of suffering AD. Additionally, e4Risk® can provide relevant information to the clinician during the diagnostic process. *APOE* ε4 carriership determination in patients with cognitive impairment can increase diagnostic precision and accelerates diagnosis, reducing diagnostic uncertainty time[8]. *APOE* ε4 is a common stratification factor in AD clinical trials and therefore, e4Risk can be used as a fist screening test to select *APOE* ε4 carriers to be included in AD clinical trials[9, 10]. Furthermore, there are evidences that *APOE* ε4 carriers respond differently to experimental [9] and currently used medication [11] , so e4Risk® could be helpful to select the dose or type of medication.

ANALYTICAL PERFORMANCE

Technical range: ~1.22-146 μg/mL

LoB*/LoD*: 0.87 μg/mL / 1.22 μg/mL

Prozone effect: 146 μg/mL still give positive result.

Interferences*: Bilirubin (up to 18 mg/mL), Human hemoglobin (up to 320 mg/dL), lipids-intralipid (≤ 2.6 g/mL), Rheumatoid factor (up to 600 UI/mL), HAMA (up to 40 ng/mL), do not interfere.

Recommended Cut-off value: 4.62 μg/mL.

Precision (QC control high):

Mean: 7.12 μg/mL

	SD	*CV(%)*
Repeatibility	0.28	3.88
Within-Laboratory	0.82	11.57

***** Determined consistent with the Guidelines in Clinical and Laboratory Standards Institute (CLSI) document EP17-A2 and with proportions of false positives (α) less than 5% and false negatives (β) less than 5%; based on 192 determinations with 96 blank and 96 low level replicates.

** Other substances may interfere

NOTES

1. This method may be used with different chemistry analyzers. Any application to an instrument should be validated to demonstrate that results meet the performance characteristics of the method. It is recommended to periodically validate the instrument. Contact the distributor for any questions on the application method.
2. Clinical diagnosis should not be made on findings of a single test result but should integrate both clinical and laboratory data.
3. For automatic instruments, avoid the presence of bubbles in the reagents that may interfere with the assay results.
4. For information regarding the methodology, see Calero O. et al (2018)[12]

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