Instructions for Use

For Research Use Only. Not for use in diagnostic procedures

AroCell TK 210 ELISA

Thymidine Kinase 1 (TK1)
Enzyme Linked Immuno Sorbent Assay

Reagents for 96 Determinations

The Instructions for Use is available to download from:
www.e-labeling.eu/ARO1002-15-2
+1 855 236 0910 The use of a landline is recommended

CE

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INTENDED USE

The AroCell TK 210 ELISA kit is a quantitative immunoassay for the determination of Thymidine Kinase 1 (TK1) in human serum and lithium-heparin plasma.

ASSAY PRINCIPLE

The AroCell TK 210 ELISA kit is a quantitative enzyme immunoassay. The test procedure is based on the sequential addition of sample, a biotin labelled anti-TK1 monoclonal antibody, streptavidin labelled enzyme-conjugate and substrate to Microtiter wells coated with monoclonal anti-TK1 IgG. The resultant color intensity is proportional to the amount of TK1 present in the sample. The Calibration Curve covers approximately 0-20 µg/L and the total assay incubation time is 4 hours and 45 minutes.

COMPONENTS

ITEM	QUANTITY
Coated Microtiter Plate with MAb anti-TK1, READY FOR USE	96 wells: 12 x 8-well strips
Sample Dilution Buffer, LYOPHILIZED	3 vials
Calibrators (CAL A-E), LYOPHILIZED	5 calibrators
Controls, LYOPHILIZED	2 controls
Wash Buffer Tablets, PBST in a sachet	3 pcs
Biotinylated MAb anti-TK1, LYOPHILIZED	1 vial
Reagent Buffer for reconstitution of the lyophilized biotinylated Mab, READY FOR USE	1 vial, 14 mL
Streptavidin-HRP conjugate, READY FOR USE	1 vial, 14 mL
TMB substrate (tetramethylbenzidine), READY FOR USE	1 vial, 14 mL
Stop Solution, 1N HCl, READY FOR USE	1 vial, 14 mL
Calibrators and Controls reference sheet	1

The kit can be used on three separate occasions.

PRECAUTIONS

SAFETY

- For Research Use Only.
- The AroCell TK 210 ELISA kit is intended for use by qualified laboratory staff only.
- The kit contains material of human origin, which has been tested and found to be negative for HIV, HCV, Hepatitis B and HTLV. However, since no test can provide complete assurance, treat all materials as potentially infectious.
- The Stop Solution contains hydrochloric acid, which is corrosive. Avoid contact with the skin and eyes. If contact occurs, rinse off immediately with water and seek medical advice.
- The Substrate contains TMB, which may irritate the skin and mucous membranes. Any Substrate, which comes in contact with the skin, should be rinsed off with water.
- Dispose of all clinical specimens, infected or potentially infected material in accordance with good laboratory practice. All such materials should be handled and disposed of as though potentially infectious.
- Residues of chemicals, preparations and kit components are generally considered as hazardous waste. All such materials should be disposed of in accordance with established safety procedures.
- Wear protective clothing, disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
- Do not pipette materials by the mouth and never eat or drink at the laboratory work bench.

PROCEDURAL

- Do not use kit or individual reagents past their expiry date.
- Do not mix or substitute reagents from different kit lot numbers.
- Deviation from the protocol provided may cause erroneous results.
- Performing the assay outside the time and temperature ranges provided may produce invalid results. Assays not falling within the established time and temperature ranges must be repeated.
- Care must be taken not to contaminate components and always use fresh pipette tips for each sample and component.
- Do not use reagents that are cloudy or that have precipitated out of solution.
- Ensure that the Wash Tablets are thoroughly dissolved and that no crystals remain after reconstitution.

Clinical Laboratory Reagent Quality water is required for reconstituting the reagents. The use of poor quality or contaminated water may lead to inaccurate results.

STABILITY AND STORAGE

The AroCell TK 210 ELISA kit can be stored at 2-8°C until the expiry date stated on the outer label of the kit.

All unopened kit reagents shall be stored at 2-8°C and are stable as supplied until the expiry date shown on the outer box label. Opened / Reconstituted components can be stored as follows:

COMPONENT	STORAGE AFTER OPENING
Coated Microtiter Plate	At 2-8 °C in plate pouch with the desiccant. Until expiry date
Calibrators and Controls	Stable for one month at –20 °C
Biotinylated MAb anti TK1	Stable for one month at 2-8 °C
Reagent Buffer	Stable at 2-8 °C until expiration date
Sample Dilution Buffer	Do not store reconstituted buffer. Use within 4 hours.
Streptavidin HRP Conjugate	Stable at 2-8 °C until expiration date
TMB substrate solution	Stable at 2-8 °C until expiration date
Stop Solution	Stable at 2-8 °C until expiration date
Wash Buffer	Tablets are stable at 2-8 °C until expiration date. Prepared wash buffer can stored one month at room temperature

ADDITIONAL MATERIALS REQUIRED

- Clinical Laboratory Reagent Quality De-ionized/Distilled water
- Adjustable micropipette, 50 - 100µL, 200 - 1000 µL and a multi-channel pipette (100 µL)
- Pipettes 5 and 12 mL
- 1L beaker
- Graduated cylinder 500 mL
- Vortex mixer
- Uncoated microtiter plate
- 4 Plate seals
- Plate shaker
- Microtiter strip washing system
- A Microtiter plate photometer capable of measuring at 450 nm
- Timer

SAMPLE COLLECTION AND HANDLING

The AroCell TK 210 ELISA kit is designed for the use with serum or lithium-heparin plasma samples. For the assay of TK1 in other types of sample matrix, contact AroCell for advice. Blood should be collected by venipuncture and allowed to clot (e.g. leave to stand at 25 °C for 30 minutes and then separate the serum by centrifugation). Lipemic or hemolysed samples should not be tested. If not assayed immediately, samples can be stored at 4 °C for up to 5 days or at –20 °C for up to 2 months. For longer-term storage, -80 °C is recommended. Avoid repeated freeze-thaw cycles. Mix samples well before testing. All samples should be assayed in duplicate.

A minimum of 180 µL of sample is required for each assay.

Samples found or expected to contain more than 15 µg/L should be diluted 1+1 with Calibrator A prior to the pre-incubation step described below.

ASSAY PROCEDURE

PREPARATION OF REAGENTS

Take the kit from the refrigerator and allow components to equilibrate to room temperature for 30 minutes.

1. Wash Buffer: Dissolve each tablet in 500 mL clinical laboratory reagent quality water. Ensure that all the salt crystals are dissolved
2. Calibrators and Controls: Reconstitute each vial in 0.75 mL clinical laboratory reagent quality water. Allow to stand for 15 minutes, and then mix the contents of the vials gently. See the Calibrators and Controls reference sheet for exact values. The Sample Dilution Buffer serves as the 0 µg/L Calibrator.
3. Sample Dilution Buffer: Reconstitute with 5 mL of clinical laboratory reagent quality water. Allow to stand for 15 minutes, and then mix contents gently. Use within 4 hours. Gently mix the Sample Dilution Buffer before use.
4. Biotinylated anti-TK1: Reconstitute with 12.0 mL of Reagent Buffer. Allow to stand for 15 minutes, then mix the contents of the vials gently.

PRE-INCUBATION

1. Dispense **80 µL** of Sample Dilution Buffer (Calibrator 0), the Calibrators A-E, Controls and Samples, including Diluted Samples from above, in duplicate into an uncoated microtiter plate.
2. Dispense **80µL** of Sample Dilution Buffer into all wells including the 0 Calibrator.
3. Mix the plate by placing briefly on an orbital shaker at intermediate speed.
4. Cover with a plate seal and incubate for **1 hour at room temperature without shaking**.

IMMUNOASSAY PROCEDURE

1. Remove the anti TK1 coated strips that will not be used in the current run from the microtiter plate and put them back in the plate pouch. Close the pouch and store at 2-8°C.
2. Wash the microtiter strips that will be used in the current run 4 times with **350 µL** Wash Buffer / well. Proceed directly to the next step which must start within 10 minutes.
3. Transfer **100 µL** of diluted Calibrators, Controls and Samples to the coated microtiter plate. The use of a multi-channel pipette is recommended.
4. Cover with a plate seal and incubate at room temperature (25°C) for **2 hours at intermediate speed on an orbital or linear shaker.**
5. Remove plate seal and wash each strip 4 times with **350 µL** Wash Buffer / well.
6. Add **100µL** Biotinylated anti-TK1 to each well.
7. Cover with a plate seal and incubate at room temperature (25°C) for **1 hour at intermediate speed on an orbital or linear shaker.**
8. Remove plate seal and wash each strip 4 times with **350 µL** Wash Buffer / well.
9. Add **100µL** Streptavidin-HRP conjugate / well.
10. Cover with a plate seal and incubate at room temperature (25°C) for **30 minutes at intermediate speed on an orbital or linear shaker.**
11. Remove plate seal and wash each strip 4 times with **350 µL** Wash Buffer / well.
12. Add **100 µL** TMB Substrate/well and **incubate stationary at room temperature in the dark for 15 minutes exactly.**
13. Add **100 µL** Stop Solution/well. Ensure complete mixing of Substrate and Stop Solution.
14. Read within 15 minutes at 450nm.

CALCULATION OF RESULTS

1. Calculate the mean absorbances for each Calibrator, Control and Sample duplicate.
2. Plot a Calibration curve of A_{450nm} versus [TK1] (µg/L) using the Calibrator values from the Calibrators and Controls reference sheet. It is recommended to use 4-parameter logistic regression (4-PL) to fit the absorbance data to a Calibration curve.
3. Read the [TK1] (µg/L) indicated by the mean absorbances of the Controls and Samples from the Calibration curve.
4. Concentrations of samples with readings above the Calibration curve should be repeated after dilution 1+1 with Calibrator A

QC CRITERIA

Controls must always be included to assess the validity of the test results. The assay is considered valid if the values of the controls are within the range given in the Calibrators and Controls reference sheet. If this criterion is not met, the assay should be considered invalid.

PERFORMANCE CHARACTERISTICS

CALIBRATION CURVE

The Calibration curve range covers approximately 0-20 µg/L (see quoted values for Calibrators in the Calibrators and Controls Reference Sheet). This range may be extended by increasing sample dilution.

LIMIT OF DETECTION

The detection limit of AroCell TK 210 ELISA kit is 0.19 µg/L based on five samples assayed in 12 different runs.

SPECIFICITY

No cross reaction has been shown with the AroCell TK 210 antibodies with Thymidine Kinase 2 (TK2) nor with murine, feline or canine TK1.

DILUTION-RECOVERY

Diluting five samples with high levels of TK1 1/2 to 1/8 with Calibrator A gave a mean recovery of 95.4%.

INTERFERING SUBSTANCES

Based on spiking four samples; No effect was seen with bilirubin concentrations up to 20mg/dL and glycerol triolate up to 300 mg/dL. Mean recovery in the presence of 60mg/mL HSA was 95% and for 400 mg/dL hemoglobin 108%.

PRECISION

The precision of the AroCell TK 210 ELISA kit was found to be as follows, based on 44 assays, each Control and Calibrator being assayed in duplicate

SAMPLE	TK1 µg/L	WITHIN ASSAY CV %	BETWEEN ASSAY CV %	TOTAL CV %
Control 1	1.85	4.9	0.9	5.1
Control 2	11.53	1.9	2.2	3.0

EXAMPLE OF A CALIBRATION CURVE

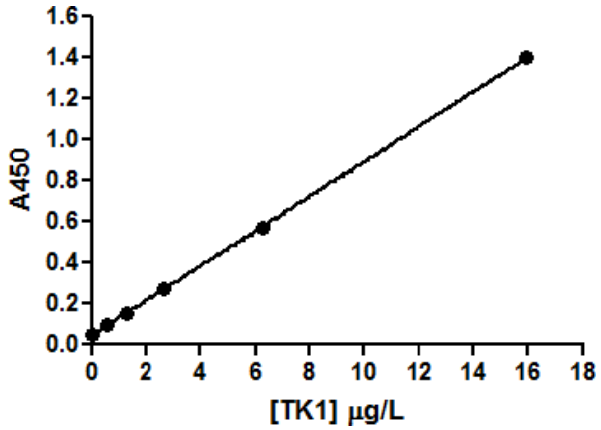
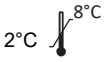


Figure 1: Example of a Calibration curve obtained using the AroCell TK 210 ELISA kit. Plot of A_{450nm} versus [TK1] µg/L. Plotted using 4-parameter Logistic Regression. This is for illustration only: Do not use for the calculation of samples.

WARRANTY

The performance data presented here was obtained using the procedure described. Any change or modification of the procedure not recommended by AroCell may affect the results, in which case AroCell disclaims all warranties, expressed, implied or statutory, including implied merchantability and fitness for use. In the case of such an event, AroCell shall not be liable for damages, direct or consequential.

INTERPRETATION OF SYMBOLS

	Consult Instructions for Use		Use by date
RUO	For Research Use Only		Manufacturer
LOT	Batch code		Contains sufficient for
REF	Catalogue Number	RCNS	Reconstitute in
	Temperature limitation	CONT	Contain

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