

AroCell TK 210 ELISA

Thymidine Kinase 1 (TK1)

Enzyme Linked Immuno Sorbent Assay

Reagents for 96 Determinations



Material Safety Data Sheets and these Instructions for Use available at:

eIFU.arocell.com

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The use of a landline is recommended

EU:

IVD



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For Research Use Only. Not for use in diagnostic procedure

TABLE OF CONTENTS

INTENDED USE3

BACKGROUND3

ASSAY PRINCIPLE.....4

COMPONENTS.....4

PRECAUTIONS5

STABILITY AND STORAGE.....6

ADDITIONAL MATERIALS REQUIRED6

SAMPLE COLLECTION AND HANDLING6

ASSAY PROCEDURE7

CALCULATION OF RESULTS8

QC CRITERIA8

PERFORMANCE CHARACTERISTICS8

EXAMPLE OF A CALIBRATION CURVE9

WARRANTY9

INTERPRETATION OF SYMBOLS9

REFERENCES10

REVISION HISTORY10

INTENDED USE

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

BACKGROUND

Thymidine Kinase 1 (TK1)

Thymidine kinase 1 phosphorylates thymidine to thymidine-monophosphate enabling subsequent incorporation into DNA. The TK1 concentrations in the cell are low in the G0/G1 phase (resting phase) of the cell cycle but increase during the S/G2 phases, when DNA synthesis occurs, and then decrease during mitosis¹.

The presence of TK1 in cells is thus an indicator of active cellular proliferation.

Sustained proliferation is a hallmark of cancer. Up-regulation of TK1 occurs during cancer development and elevated TK1 levels have been reported also in pre-cancerous conditions². Increased TK1 expression is often associated with increased expression of other cell proliferation markers such as the Ki-67 antigen and proliferating cell nuclear antigen (PCNA) although studies have shown that TK1 may be more useful as a proliferation marker than either of them³.

TK1 in serum

Serum TK1 enzyme activity has been shown to be elevated in subjects with many forms of cancer, including leukemia, lymphoma, prostate, breast, lung, sarcoma and colon cancer patients⁴. Measuring TK1 in serum is a useful complement to immunohistological testing with proliferation biomarkers and has a practical advantage in simplifying serial testing.

Some studies found that, together with traditional tumor biomarkers (e.g. CA 15-3), TK1 activity indicated cell proliferation and turnover rate, while others were related to tumor mass⁵.

High pre-treatment serum TK1 activities have been associated with shorter progression-free and overall survival in subjects with breast cancer⁵. Conversely, subjects with cancer but with low serum TK1 values often show improved survival⁶. Continued elevations in serum TK1 following surgery may indicate residual tumor cells and an increased risk of disease progression.

Thymidine Kinase 1 (TK1) has been used as a valuable biomarker for cellular proliferation since 1980, however, previous methods were based on enzyme activity measurements that may be subject to interference⁷ and may underestimate TK1, especially in serum from subjects with solid tumors.

Furthermore, serum TK1 occurs as aggregates with a range of molecular weights and specific activities and this distribution differs between serum from healthy subjects and those with tumors⁸.

The AroCell TK 210 ELISA kit procedure includes pre-treatment of the samples with a Sample Dilution Buffer that makes TK1 aggregates more readily accessible for immunoassay. The AroCell TK 210 ELISA kit brings the specificity and sensitivity of immunoassay to the assay of TK1 and offers improved accuracy, especially when studying serum TK1 derived from patients with solid tumors^{9,10}. A study comparing TK1 levels in healthy and breast cancer subjects showed the AroCell TK 210 ELISA to better distinguish between these groups than an enzyme activity assay⁹. Recent studies showed that TK 210 ELISA can predict the overall survival in prostate cancer alone or combination with PSA levels^{11,12}.

Another study demonstrated the application of TK 210 ELISA in early detection of Ovarian cancer¹³. The TK 210 ELISA kit provides new opportunities for studying cellular proliferation, tumor cell turnover, and therapy response¹⁴.

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 ng/mL 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	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

- 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 workbench.

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 samples. For the assay of TK1 in other types of sample matrix, contact AroCell for advice. Blood should be collected by venepuncture 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 ng/mL should be diluted 1+1 with Calibrator A prior to the pre-incubation step described below.

ASSAY PROCEDURE


Preparation of reagents

1. Take the kit from the refrigerator and allow components to equilibrate to room temperature for 30 minutes.
2. Wash Buffer: Dissolve each tablet in 500 mL clinical laboratory reagent quality water. Ensure that all the salt crystals are dissolved.
3. 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 ng/mL Calibrator.
4. Sample Dilution Buffer: Reconstitute with 5 mL of clinical laboratory reagent quality water. Allow to stand for 15 minutes, and then mix the contents gently. Use within 4 hours. Gently mix the Sample Dilution Buffer before use.
5. 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 μ L 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 80 μ L 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 μ L Wash Buffer / well. Proceed directly to the next step which must start within 10 minutes.
3. Transfer 100 μ L 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 μ L Wash Buffer / well.
6. Add 100 μ L 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 μ L Wash Buffer / well.
9. Add 100 μ L 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 μ L Wash Buffer / well.
12. Add 100 μ L TMB Substrate/well and incubate stationary at room temperature in the dark for 15 minutes exactly.
13. Add 100 μ L 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] (ng/mL) 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] (ng/mL) 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 ranges given in the Calibrators and Controls reference sheet. If this criterion is not met, the assay should be considered invalid.

PERFORMANCE CHARACTERISTICS

Reference range

Based on data from 366 apparently healthy blood donors, the 95% percentile was 0.40 ng/ mL_{10, 15}. However, AroCell recommends that all laboratories determine their own reference ranges.

Calibration curve

The Calibration Curve range covers approximately 0-20 ng/mL (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 0.12 ng/mL₁₅

Sixty replicates of SDB measurements over 4 independent assays were used to calculate the LOB (95.5th percentile). To determine the limit of detection (LOD), 5 serum samples with TK1 protein levels in the range of 0.3 to 1.0 ng/mL were used. Sixty replicates were measured over 12 independent assays and the LOD was calculated: $LOD = LOB + 1.653 * SD$ (Standard deviation of the serum sample measurements).

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%₁₅.

Interfering substances

Based on spiking four samples; No effect was seen with bilirubin concentrations up to 0.2mg/mL and glyceryl triolate up to 3 mg/mL₁₅. Albumin levels below 60mg/mL did not significantly affect results. The assay of hemolyzed samples is not recommended.

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 ng/mL	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

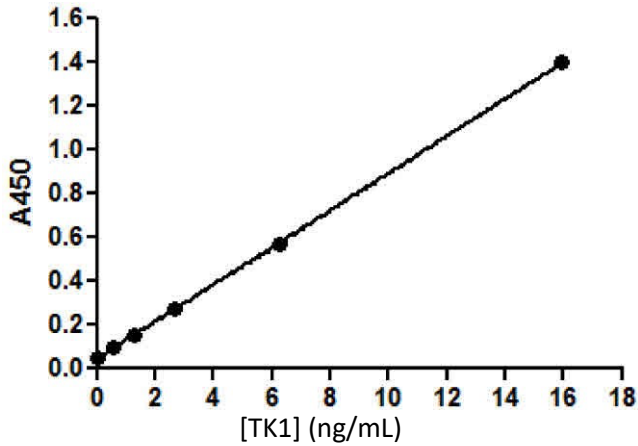


Figure 1: Example of a Calibration Curve obtained using the AroCell TK 210 ELISA kit. Plot of A_{450nm} versus [TK1] ng/mL. Plotted using 4-parameter Logistic Regression. This is for illustration only: Do not use for the calculation of samples.

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



In vitro diagnostic Medical Device



Use by date:



For Research Use Only



Manufacturer:



Batch code



Contains sufficient for <n> tests



Catalogue Number



Temperature limitation

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REVISION HISTORY

Ver.	Date	Description of the change
01	2024-08-28	Page 1: AroCell IFU (31-11TF70B1-09) has been updated with new REF number 91-124 version 1 to compile with IDL Biotech QMS system. Page 9: The symbols for reconstitute in and contains has been removed from the IFU text. Page 10: New references (reference 11-15) have been included Page 11: This version has also been updated with new address



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