AroCell TK 210 ELISA

Thymidine Kinase 1 (TK1) Enzyme Linked Immuno Sorbent assay

Reagents for 96 Determinations

Catalog Number: 139959 (1 x 96 Wells)

Instructions for Use available to download from: www.e-labeling.eu/ARO1001-15
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USA: For Research Use Only

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INTENDED USE
The AroCell TK 210 ELISA Assay kit is a quantitative immunoassay for the determination of Thymidine Kinase 1 (TK1) in human serum. The TK 210 ELISA Assay kit is For Research Use Only. The performance characteristics of this product have not been established.

BACKGROUND

Thymidine Kinase 1
Thymidine kinases phosphorylate thymidine to thymidine-monophosphate enabling subsequent incorporation into DNA. Thymidine Kinase 1 (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 mitosis. 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 conditions. 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 them.

TK1 in serum
95% of serum TK1 activity in cancer positive samples is apparently tumor derived, making TK1 an excellent indicator of proliferation and cell turnover. 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 patients. 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 that of traditional tumor biomarkers (e.g. CA 15-3), TK1 activity provided an indication of the rate of cell proliferation and cell turnover, while other markers were related to tumor mass.

High pre-treatment serum TK1 activities have been associated with shorter progression-free and overall survival in subjects with breast cancer. Conversely, subjects with cancer but with low serum TK1 values often show improved survival. 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 interference 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 tumors.

The AroCell TK 210 ELISA Assay 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 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. 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 assay. The AroCell TK 210 ELISA will provide new opportunities for studying cellular proliferation, tumor cell turnover and therapy response in subjects with solid tumors.
ASSAY PRINCIPLE

The AroCell TK 210 ELISA Assay kit is a quantitative enzyme immunoassay. The test procedure is based on the sequential addition of sample, a biotin labeled anti-TK1 monoclonal antibody, streptavidin labeled enzyme-conjugate and substrate to Microtiter wells coated with another monoclonal anti-TK1 IgG. The resultant color intensity is proportional to the amount of TK1 present in the sample. The assay range is approximately 0 - 17 μg/L and the total assay incubation time is 4 hours and 45 minutes.

COMPONENTS

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

The kit can be used on three separate occasions.
PRECAUTIONS

SAFETY

- The TK 210 ELISA Assay kit is For Research Use Only. The performance characteristics of this product have not been established.
- 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:

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

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 is designed for the use with serum samples. For the assay of TK1 in other types of sample, contact AroCell / Eagle Biosciences 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 hemolyzed 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.

Samples found or expected to contain more than 17 μg/L should be diluted 1+4 with serum dilution buffer 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 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.
4. Sample dilution buffer: Reconstitute with 5 mL of clinical laboratory reagent grade water. Allow to stand for 15 minutes, and then mix contents gently. Use within 4 hours. Gently mix the reagent buffer before use.
5. Biotinylated anti-TK1: Reconstitute with 12.0 mL of the 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 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. Wash the anti TK1 coated microtiter plate 4 times with 350 μL wash buffer. Proceed directly to the next step which must start within 10 minutes.
2. Transfer 100 μL of diluted calibrators, controls and samples to the coated microtiter plate. The use of a multi-channel pipette is recommended.
3. Cover with a plate seal and incubate at room temperature (25°C) for 2 hours at intermediate speed on an orbital or linear shaker.
4. Remove plate seal and wash each strip 4 times with 350 μL Wash Buffer.
5. Add 100μL Biotinylated anti-TK1 to each well.
6. Cover with a plate seal and incubate at room temperature (25°C) for 1 hour at intermediate speed on an orbital or linear shaker.
7. Remove plate seal and wash each strip 4 times with 350 μL Wash Buffer.
8. Add 100μL streptavidin-HRP conjugate / well.
9. Cover with a plate seal and incubate at room temperature (25°C) for 30 minutes at intermediate speed on an orbital or linear shaker.
10. Remove plate seal and wash each strip 4 times with 350 μL Wash Buffer.
11. Add 100 μL TMB Substrate/well and incubate stationary at room temperature in the dark for 15 minutes exactly.
12. Add 100 μL Stop Solution/well. Ensure complete mixing of Substrate and Stop Solution.
13. 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.
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 standard curve should be repeated after dilution.

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

REFERENCE RANGES
Kumar et al\(^\text{10}\) reported a reference range (logarithmic mean ± 1SD) of 0 – 0.35 μg/L, based on a population of 53 healthy blood donors. However, AroCell recommends that all laboratories determine their own reference ranges.

LIMIT OF DETECTION
The detection limit of AroCell TK 210 ELISA is 0.17 μg/L based on five samples assayed in 12 different runs.

MEASURING RANGE
The calibration curve range covers approximately 0 - 17 μg/L (see quoted values for Calibrators in the Calibrators and Controls Reference Sheet). This range may be extended by increasing sample dilution.

DILUTION-RECOVERY
Diluting three samples with high levels of TK1 with serum samples with low levels of TK1 showed less than 5% deviation from linearity.

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

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>TK1 μg/L</th>
<th>WITHIN ASSAY CV %</th>
<th>BETWEEN ASSAY CV %</th>
<th>TOTAL CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>1.85</td>
<td>4.9</td>
<td>0.9</td>
<td>5.1</td>
</tr>
<tr>
<td>Control 2</td>
<td>11.53</td>
<td>1.9</td>
<td>2.2</td>
<td>3.0</td>
</tr>
</tbody>
</table>
EXAMPLE OF CALIBRATION CURVE

![Calibration Curve Image]

**Figure 1:** Typical Calibration curve obtained using the AroCell TK 210 ELISA. Plot of $A_{450}$ versus [TK1] μg/L. Assay range is 0 - 17 μg/L TK1. 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**

- **i**: Consult Instructions for Use
- **IVD**: *In vitro* diagnostic medical device
- **RUO**: For Research Use Only Batch
- **LOT**: Code
- **REF**: Catalogue Number
- **°C**: Temperature limitation
- **Use by date**: Use by date
- **Manufacturer**: Manufacturer
- **Contains sufficient for**: Contains sufficient for
- **Reconstitute in**: Reconstitute in
- **Contains**: Contains
REFERENCES


