

TPAcyk™ ELISA

REF 10-023

INSTRUCTIONS FOR USE

INTENDED USE

TPAcyk™ ELISA is a one step assay for the determination of cytokeratin 8 and 18 in serum. The assay is a sensitive indicator of tumor cell activity and is useful in the management of patients with carcinomas of epithelial origin. This assay is for research use only, not for use in diagnostic procedures.

PRINCIPLE OF THE ASSAY

TPAcyk™ ELISA is a solid phase sandwich assay based on immunochemical technique. Standards, controls and samples react simultaneously with solid phase catcher antibodies (6D7 and 3F3) and the HRP conjugated detector antibody during incubation in the microstrip wells. After washing, the TMB substrate is added. Subsequently the reaction is stopped and the absorbance is read. The developed color is directly proportional to the concentration of the analyte.

ASSAY SPECIFICITY

TPAcyk™ measures key epitopes of TPA (Tissue Polypeptide Antigen) fragments. The monoclonal 6D7 and 3F3 antibodies used in the test are specific for cytokeratin 8 and 18, with no detectable cross reactivity to other tumor associated antigens that may be present in patient samples.

SAMPLES

Serum samples are recommended. Enough blood should be collected to be sufficient for 2 x 100 µl serum (duplicates) at each analysis. If the analysis will be performed within 24 h, the serum should be refrigerated (2-8 °C). If delayed analysis, serum should be frozen in aliquots (<-18 °C). Avoid repeated thawing and freezing. Do not use serum samples that are grossly lipemic, hemolysed or contaminated.

PRECAUTIONS FOR USERS

1. TPAcyk™ ELISA is for research use only, not for use in diagnostic procedures.
2. Wear protective gloves and protective goggles.
3. Do not use the kit after expiry date.
4. Do not mix reagents from different lots.
5. All patient samples should be regarded as contagious and handled and disposed of according to appropriate regulations.
6. Avoid microbiological contamination of reagents.
7. Analysis should be performed according to GLP.
8. The accuracy of the test is related to adherence to the assay procedure and accurate volume pipetting.
9. The Stop Solution contains 0.5 M sulfuric acid, which might cause irritation on skin, and is harmful to eyes. In case of contact flush with plenty of water and seek medical advice.
10. The TMB Substrate might cause irritation on skin and eyes. In case of contact flush with plenty of water and seek medical advice.
11. ProClin 300 (60 ppm) used as preservative in this product might be allergenic. In case of contact flush with plenty of water and seek medical advice.
12. Material Safety Data Sheets are available on request.

MATERIALS REQUIRED BUT NOT PROVIDED

Microplate reader (wavelength 450 nm).
Microplate shaker (oscillation ~450 rpm).
Microplate wash equipment.
Routine laboratory equipment, e.g. precision pipettes and vortex.

COMPONENTS IN THE TPAcyk® ELISA

Materials supplied for 96 determinations.

TPAcyk™ Coated Microstrips: 1 plate, 96 dry wells (12x8), coated with monoclonal anti-cytokeratin 8/18 antibodies (6D7 and 3F3). Packed in aluminum bag with desiccating device. Ready for use.

TPAcyk™ ELISA HRP Conjugate: 2 vials, 0.5 ml/vial, conjugated antibody in protein stabilized buffer, pH 7.5 (11 x conc.). Should be diluted with TPAcyk™ Diluent (Standard 0 ng/ml). Blue colored. Preservative added.

TPAcyk™ Diluent (Standard 0 ng/ml): 1 vial, 15 ml, sample diluent and standard 0 ng/ml, protein stabilized buffer, pH 7.5. Preservative added. Ready for use.

TPAcyk™ ELISA Standard (1, 2, 5, 10, 15 ng/ml): 5 vials standard, 1 ml/vial, TPAcyk™ standard material in protein stabilized buffer, pH 7.5. Concentrations as stated on vials. Preservative added. Ready for use.

TPAcyk™ Control (Low, High): 2 vials control (1 ml/vial), Lyophilized Controls, TPAcyk™ standard material in protein stabilized buffer, pH 7.5. Yellow colored. Preservative added.

Wash Tablet: 1 package, 1 tablet/package. The tablet should be dissolved in 500 ml fresh deionized water.

TMB Substrate: 1 vial, 22 ml. Protect from light and keep lid tightly closed. Do not sample more than what is needed for the analysis. Ready for use.

Stop Solution: 1 vial, 12 ml, 0.5 M sulfuric acid. Ready for use.

Sealing Tape: 1 sheet, Sealing tape for microstrips.

TPAcyk™ ELISA Certificate: 1 protocol. Certificate of lot content.

ASSAY PROCEDURE

The assay (see Flow chart) should be performed at room temperature (RT; 22 ± 6 °C).

1. Allow all reagents and samples to adjust to RT. Vortex all reagents prior to use.
2. Dilute the TPAcyk™ Control (Low, High) with 1.0 ml fresh deionized water. Let vials stand, mix thoroughly after 10 min. Ready to use 15 min after reconstitution.
3. Based on the number of strips needed, dilute TPAcyk™ ELISA HRP Conjugate (11 x conc.) with TPAcyk™ Diluent (5.0 ml/vial). Mix thoroughly.
4. Pipette 100 µl standards, controls or samples per well (duplicates). Start with two empty wells for background absorbance measurement (blank).
5. Add 100 µl diluted HRP Conjugate to each well, except the two empty wells. Cover the strips with the supplied Sealing Tape. *NB! Steps 4 and 5 should be performed sequentially without interruption.*
6. Incubate for 2 h ± 2 min on shaker (~450 rpm).
7. Prepare the wash solution. Dissolve Wash Tablet in 500 ml fresh deionized water.
8. Aspirate and wash the wells 3x0.3 ml with wash solution.
9. Add 200 µl TMB Substrate to each well, including the two empty wells. Incubate in darkness for 15 ± 1 min.
10. Add 100 µl Stop Solution to each well. Agitate on shaker 1 min (~450 rpm).
11. Read the absorbance at 450 nm, within 30 min after addition of the Stop Solution.
12. Calculate the cytokeratin 8 and 18 concentration (ng/ml) of the samples. Samples showing concentrations >15 ng/ml value should be suitably diluted with TPAcyk™ Diluent (Standard 0 ng/ml) before repeated analysis.

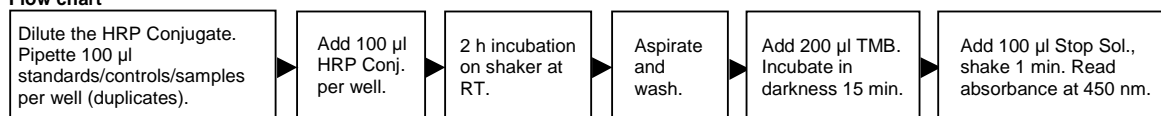
PROCESSING OF RESULTS

Manual calculation or by using a computer software for handling ELISA-type data (curve fitting - Spline smoothed). For generation of valid data, ensure that included controls are within range. *Manual processing of results:* Correct each absorbance value by subtracting the background absorbance (blank). Estimate the mean value for each duplicate. Construct a standard curve by plotting the mean absorbance value for each standard (y-axis) against the corresponding concentration (x-axis). Determine the concentrations of the samples against the standard curve.

REAGENT STORAGE

The kit should be stored at 2-8 °C. **Do not freeze!** Store reagents in their original containers if not used at once. Reseal the Microstrip bag, including the desiccating device, if not all strips are used at once. The wash solution is stable for 4 weeks when stored at 2-8 °C. The diluted TPAcyk™ ELISA HRP Conjugate is stable for 4 weeks when stored at 2-8 °C. The reconstituted controls are stable for 4 weeks when stored at 2-8 °C.

Flow chart



LIMITATIONS OF THE PROCEDURE

The test is for research use only, not for use in diagnostic procedures. The assay values should be interpreted in conjunction with all available information. Increased values can also be found e.g. in cases of pregnancy, liver disease, renal failure and general infections. If a temporary infection is suspected, it may be necessary to repeat the test two weeks later.

ASSAY CHARACTERISTICS

Measuring range: The measuring range is 0-15 ng/ml. The assay has no "high-dose hook effect" up to 500 ng/ml.

Reproducibility: The intra-assay reproducibility of the standard curve has a typical CV of <5 % (in duplicates). Inter-assay imprecision is characteristically of 5-10 % CV.

Sensitivity: The minimal detectable concentration in TPAcyk™ ELISA is 0.1 ng/ml, defined as the concentration of TPA that corresponds to the absorbance being three standard deviations from the absorbance of standard 0 ng/ml.

Expected values: The 95th percentile for apparently healthy individuals (Swedish blood donors) has been determined to 1.0 ng/ml. Due to differences which may exist between laboratories and locales with respect to population, laboratory technique and selection of reference groups, it is recommended that each laboratory establishes its own normal range.

Recovery: Specified quantities of TPAcyk™ were added to human serum samples. The recovery was 88-98 %.

WARRANTY

The performance data presented here were obtained using the procedure indicated. Any change or modification in the procedure, not recommended by IDL Biotech AB, may affect the results. In such event IDL Biotech AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and the fitness for use.

REFERENCES

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3. Sütterlin, M. *et al.* Clinical value of CYFRA 8/18 and TPS in the diagnosis and follow up of invasive breast cancer. *Anticancer Res* 1997; 17:2963-2966.

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