CE

ENGLISH

TPAcyk™ IRMA

REF 10-021

INSTRUCTIONS FOR USE

INTENDED USE

TPAcyk™ IRMA 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™ IRMA is a solid phase radiometric sandwich assay based on immunochemical technique. Standards, controls and samples react simultaneously with solid phase catcher antibodies (6D7 and 3F3) and the ^{125}I-labeled detector antibody during incubation in assigned tubes. After washing, the radioactivity is assessed in a gamma counter. The radioactivity 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

A. General

1. TPAcyk™ IRMA 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 specimens 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. 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.
10. Material Safety Data Sheets are available on request.

B. Radioactive material

1. Radioactive material must be handled according to local regulations and may be received, acquired, possessed and used only by possessors of appropriate permissions.
2. Radioactive material should be stored and handled in designated areas. Immediately decontaminate spilled material. Wash all contaminated areas with a suitable detergent.
3. Do not eat, drink or smoke within the designated work area.
4. Wear protective gloves and goggles during handling of radioactive materials, wash hands thoroughly afterwards.
5. All material used should be considered as radioactive and disposed of in designated containers.

MATERIALS REQUIRED BUT NOT PROVIDED

Gamma counter for ^{125}I (efficiency > 40%).
Shaker for tubes (oscillation ~450 rpm).
Wash equipment for tubes.
Tubes and bead dispenser.
Routine laboratory equipment, *e.g.* precision pipettes and vortex.

COMPONENTS IN THE TPAcyk™ IRMA

Materials supplied for 100 determinations.

TPAcyk™ Coated Beads: 1 bottle, 100 dry beads, coated with monoclonal anti-cytokeratin 8/18 antibodies (6D7 and 3F3). Packed with desiccating device. Ready for use.

TPAcyk™ IRMA ^{125}I Tracer: 1 vial, 11 ml, ^{125}I-labeled antibody in protein stabilized buffer, pH 7.5. Radioactivity ~ 1.1 Mbq/vial. Red colored. Preservative added. Ready for use.

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™ IRMA Standard (0.5, 1, 2, 5, 15, 30 ng/ml): 6 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: 2 packages, 1 tablet/package. Each tablet should be dissolved in 500 ml fresh deionized water.

TPAcyk™ IRMA 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. Pipette 100 µl standards, controls or samples per tube (duplicates).
4. Add 1 bead per tube.
5. Add 100 µl TPAcyk™ IRMA ^{125}I Tracer to each tube. Cover the tubes with plastic film. *NB! Steps 4 and 5 should be performed sequentially without interruption.*
6. Incubate for 4 h ± 5 min on shaker (~450 rpm).
7. Prepare the wash solution. Dissolve each Wash Tablet in 500 ml fresh deionized water.
8. Aspirate and wash the tubes 3 x 3 ml with wash solution.
9. Assess the radioactivity in a gamma counter. Add two empty tubes for background cpm measurement.
10. Calculate the cytokeratin 8 and 18 concentration (ng/ml) of the samples. Samples showing concentrations >30 ng/ml value should be suitably diluted with TPAcyk™ Diluent (Standard 0 ng/ml) before repeated analysis.

PROCESSING OF RESULTS

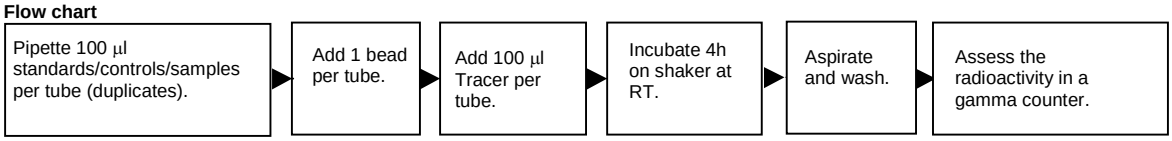
Manual calculation or by using a computer software for handling IRMA-type data (curve fitting - Spline smoothed). For generation of valid data, ensure that included controls are within range.

Manual processing of results: Correct each cpm (counts per minute) value by subtracting the background radioactivity (cpm). Estimate the mean value for each duplicate. Construct a standard curve by plotting the mean cpm 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 bottle with Coated Beads, including the desiccating device, if not all beads are used at once. The wash solution 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 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 0-30 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™ IRMA is 0.1 ng/ml, defined as the concentration of TPA that corresponds to the cpm being three standard deviations from the cpm 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 86-101 %.

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.

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