DRG® TPS (CYK18) ELISA (EIA-2354)

Revised 29 Apr. 2011 rm (Vers. 8.1) **RUO in the USA**

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

INTENDED USE

TPS ELISA is intended for determination of cytokeratin 18 in serum.

PRINCIPLE OF THE ASSAY

TPS ELISA is a one step enzyme linked sandwich immunoassay. Standards, controls and samples react during incubation simultaneously with a solid phase monoclonal catcher antibody and the HRP-conjugated detector antibody (M3). After washing, the TMB substrate is added and after an incubation time the reaction is stopped and the absorbance at 450 nm is measured. The developed colour is directly proportional to the concentration of the analyte.

SPECIMENS

Serum samples or heparinized plasma samples are recommended.
Enough blood should be collected to be sufficient for 2 x 50 µl sample (duplicates) at each analysis.
If the analysis will be performed within 24 h, the specimen should be refrigerated (2 - 8 °C). If delayed analysis, serum should be frozen (≤ -18 °C).
Avoid repeated thawing and freezing. Do not use serum samples that are grossly lipemic or contaminated. Avoid hemolyzed samples, they may cause false positive results.

PRECAUTIONS

1. Do not use the kit after expiry date.
2. Do not mix reagents from different lots.
3. The accuracy of the test is related to adherence to the assay procedure and accurate volume pipetting.
4. Standards, controls and samples in duplicates are recommended.
5. All specimens should be regarded as contagious, handled and disposed of according to appropriate regulations.
6. Wear protective gloves and eyewear.
7. Avoid microbiological contamination of reagents.
8. Do not eat, drink or smoke within the designated work area.
9. Material Safety Data Sheet is available on request.

MATERIALS REQUIRED BUT NOT PROVIDED

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Microplate reader (wavelength 450 nm)
Shaker for incubation with a recommended oscillation ~450 rpm.
Wash equipment for microplates.
Routine laboratory equipment, *e.g.* precision pipettes and vortex.
Deionized or distilled water.

COMPONENTS IN TPS ELISA

Materials supplied for 96 determinations.

TPS ELISA Microstrips
1 plate, 96 dry wells (12 strips), coated with monoclonal anti-cytokeratin 18 antibody.
Packed in aluminium bag with desiccating device. Ready for use.

TPS ELISA HRP Conjugate
1 vial, 11 ml, M3 antibodies conjugated with HRP, protein stabilized buffer, pH 7.5.
Blue colored. Preservative added. Ready for use.

TPS ELISA Diluent (Standard 0 U/l)
1 vial, 5 ml, sample diluent and standard 0 U/l, protein stabilized buffer, pH 7.5.
Yellow colored. Preservative added. Ready for use.

TPS ELISA Standard (30, 150, 500, 1200 U/l)
4 vials standard, 1 ml/vial, TPS ELISA standard material in protein stabilized buffer, pH 7.5.
Concentrations as stated on vials. Yellow colored. Preservative added. Ready for use.

TPS ELISA Control (Low, High)
2 vials, 1 ml/vial, TPS ELISA standard material in protein stabilized buffer, pH 7.5. Y
ellow colored. Preservative added. Ready for use.

Wash tablet: One blisterpacked tablet, the tablet should be dissolved in 500 ml of 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.

TPS ELISA Certificate Certificate of lot content.

CE

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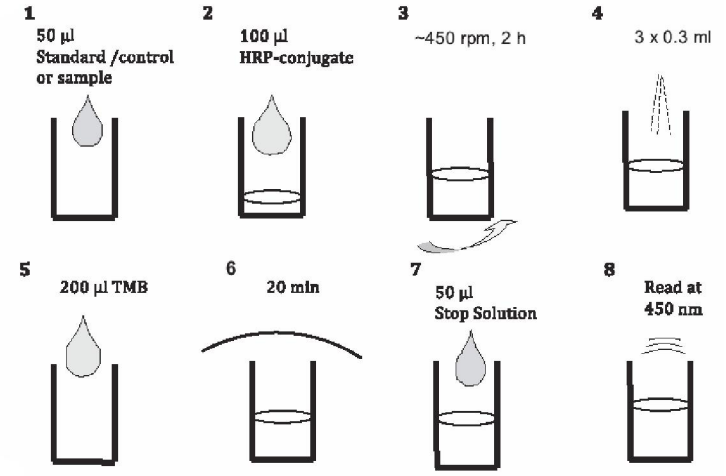
ASSAY PROCEDURE

The assay should be performed at room temperature, 22 ± 6 °C.
Allow all reagents and samples to adjust to room temperature. Vortex all reagents prior to use.

1. Pipette 50 µl standards, controls or samples per well. Leave two empty wells for background absorbance measurement (blank).
2. Add 100 µl TPS ELISA HRP Conjugate per well except the blank wells.
   Nb! Steps 1 and 2 should be performed sequentially without interruption.
3. Incubate for 2 h ± 10 min on a shaker at ~450 rpm.
   Correct setting of the shaker is important for correct results.
4. Prepare the wash solution by dissolving one wash tablet in 500 ml of deionized water.
5. Aspirate and wash the wells 3 times with 0.3 ml wash solution.
6. Add 200 µl TMB substrate per well, including the blank wells. Incubate in darkness for 20±1 min.
7. Add 50 µl Stop Solution per well. Agitate on a shaker for 1 min.
8. Read the absorbance at 450 nm within 30 min after addition of the Stop Solution
9. Calculate the cytokeratin 18 concentration (U/l) of the samples.
   Samples showing concentrations > 1200 U/l should be suitably diluted with TPS ELISA Diluent (Standard 0 U/l) before repeated analysis.

Schematic assay procedure



PROCESSING OF RESULTS

Use computer software for handling the raw data. Use Spline smoothed as a curve fitting algorithm. For generation of valid data, ensure that included controls are within range.

Manual processing of results:
Correct each OD-value (optical density) by subtracting the blank OD. Calculate the mean OD-value for each duplicate. Construct a standard curve by plotting the mean OD-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. The reagents should be stored in their original containers. Be sure to store TPS ELISA micro well strips in the aluminium bag with desiccating device, if not all strips are used at once. The wash solution is stable for 4 weeks when stored at 2-8°C.

LIMITATIONS OF THE ASSAY

The assay values should be interpreted in conjunction with all available clinical 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 at a later occasion.

WARRANTY

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

REFERENCES

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