**TECHNOZYM® ADAMTS-13 INH**

**Symbols key / Symbolschlüssel / interpretazione dei simboli / clé des symboles**

- **DIL**: dilute or dissolve in / verdünnen oder lösen in / a diluire o a sciogliere in / à diluer ou à dissoudre
- **INC**: incubation buffer / Inkubationspuffer / tampone di incubazione / tampon d’incubation
- **LOT**: lot / Charge / lotto / lot
- **MTP**: microtiter plate / Mikrotiterplatte / placa microtiter / microplaques sensibilisées
- **REF**: catalogue number / Katalognummer / numero di catalogo / référence
- **RTU**: ready to use / gebrauchsfertig / pronto all’uso / prêt à l’emploi
- **SUB**: substrate / Substrat / substrato / substrat
- **STOP**: stop solution / Stopplösung / soluzione di arresto / solution d’arrêt
- **WASH**: washing solution concentrated / Waschlösungskonzentrat / concentrado de solución de lavado / concentré de solution de lavado
**PRODUCT DESCRIPTION**

**INTENDED USE**

The TECHNOZYM® ADAMTS-13 INH ELISA is a test for detection of human autoantibodies (IgG) in serum or plasma against ADAMTS-13 protease, which is responsible for cleaving unusually large WPF (ULWPF). Most of these autoantibodies inhibit ADAMTS-13 activity and thus non-cleaved ULWPF accumulates in plasma. This is believed to be the major cause for thrombotic thrombocytopenic purpura (TTP). TECHNOZYM® ADAMTS-13 INH test makes it possible to differentiate between congenital (gene polymorphisms) and acquired (autoantibodies) TTP when coupled to an activity assay and to control efficacy of plasma exchange therapy.

**COMPOSITION**

1. ELISA test strips (12) with 96 wells each, coated with a recombimant form of ADAMTS-13 protease; the drying agent is supplied in an aluminium bag.
2. Washing buffer concentrate (PBS, pH 7.3), containing: 0.01% methanol; 1 vial, 80 mL.
3. Incubation buffer (+ sample dilution buffer) (PBS, pH 7.3), contains stabiliser proteins; 0.05% acetic acid and dyes; 1 vial, 90 mL, ready for use.
4. Calibrators (Standards) numbered from 1 to 5; lyophilised; 1 vial each; 0.5 mL.
5. Substrate solution sulphuric acid 0.45 mol/L; 1 vial; 12 mL; ready to use.
6. Multichannel and/or dispensing pipettes (100 and 200 µL).
7. Chromogenic substrate TMB (tetramethylbenzidine); 1 vial; 12 mL; ready to use.
8. Stopping solution (sulphuric acid) 2...8 °C
9. Adhesive film; for ELISA test strips; 2 pieces.

**MATERIAL REQUIRED** (not supplied with the kit)

- Diluted water
- Test tubes for diluting samples
- Measuring cylinder (1000 ml)
- Precision pipettes (10, 100 and 1000 µL)
- Variable pipettes (1500 µL)
- Multichannel and/or dispensing pipettes (100 and 200 µL)
- ELISA washer or multichannel dispenser
- ELISA reader with 450 nm filter, with a 620 nm reference filter if available.

**STABILITY AND STORAGE**

The expiry date printed on the labels applies to storage of the unopened vial at + 2 ... 8 °C

- Stability after reconstitution/opening:
  - ELISA test strip: after reconstitution at + 2 ... 8 °C with adhesive film in plastic box: 6 months
  - Washing buffer: after opening at + 2 ... 8 °C: 6 months
  - Washing buffer concentrate: after opening at + 2 ... 8 °C: 3 weeks
  - Incubation buffer: after reconstitution at + 2 ... 8 °C: 3 months
  - Incubation buffer concentrate: after opening at + 2 ... 8 °C: 3 weeks
  - Conjugate protein: after opening at + 2 ... 8 °C: 6 months
  - Chromogenic TMB: after opening at + 2 ... 8 °C: 24 months

**TEST PROCEDURE**

**PREPARATION OF THE SAMPLES**

Sample material: Human serum or diluted plasma. Samples may be stored for three hours at room temperature. At -20°C they can be stored for several months. Samples may not be frozen and thawed several times.

**PREPARATION OF REAGENT**

1. Before starting the test, all the required components are to be brought to room temperature.
2. Prepare the washing buffer: Dilute 1 part by volume washing buffer concentrate with 11.5 parts by volume distilled water (w:v). Mix well. (Diluted washing buffer concentrate = washing buffer). There may be crystalline precipitations which will dissolve at 37°C within 10 minutes.
3. Reconstitute calibrators and control plasma: Calibrators and control plasma are reconstituted with 500 µL distilled water and mixed for 10 seconds after a reconstitution time of 15 minutes (vortex mixer).
4. The calibrators and control plasma brothers are clear to slightly turbid.
4.4. Reconstituted components are clear to slightly turbid.

**Sample Dilution**

- ELISA reader, 450 nm
- Measurement of samples with high concentrations of other than anti ADAMTS-13 autoantibodies may result in weak positive or borderline results.

**Assay Results**

**QUALIFICATION OF THE RESULTS / REFERENCE RANGE**

- The extinction difference between these two values should be at least 1.0.
- The validity of the test may be checked on the basis of the calculated control values.

**Example of standard curve**

**Performance Characteristics**

Performance data are given below. Results obtained in individual laboratories may differ.

**INTERPRETATION OF RESULTS / REFERENCE RANGE**

- The extinction coefficient of the highest calibrator should be between 1.0 and 2.5.
- The extinction coefficient of the lowest calibrator should be 0.1.
- The extinction difference between these two values should be at least 1.0.
- No dilution is necessary for calibrators and controls.
- The results were obtained.

**STANDARDISATION**

Standards are calibrated against a plasma with a very high titre of anti ADAMTS-13 IgG. A 1:200 dilution of this reference plasma is defined to contain an antibody concentration of 100 units/mL (arbitrary units).

**MEASURING**

- Pipette calibrators, control plasmas, diluted samples into test wells; cover test strips with film
- Incubate at room temperature
- Incubate at room temperature
- Incubate at room temperature
- Incubate at room temperature

**REFERENCE**

1. Reagents of different lots must not be combined
2. Precision and performance, among others, essentially depend on the following factors:
- Thorough mixing of all substances used for dilution, 10 sec. with Vortex Mixer
- Test calibrators, controls and samples in duplicates
- Incubate at indicated temperature (RT: room temperature, 20...25°C)
- Strict observance of the order of pipetting and of the time element as indicated
- The time for sample incubation, conjugate and substrate reaction as indicated after pipetting the last sample. Incubation times should not vary by more than ±5 %
- During sample incubation and conjugate reaction, the time for pipetting calibrators/control plasmas and/or conjugate solutions must not exceed 80 seconds per ELISA test strip.
- During substrate reaction and at stopping, the time needed for pipetting the substrate and/or conjugate solution must not exceed 10 seconds per ELISA test strip. Short pipetting times may be secured by using multichannel- and dispensing pipettes.
- Use incubation buffer from actual kit box, do not use incubation buffer left from previous boxes.

**WARNING AND PRECAUTIONS**

**Sample management**

- All human blood or plasma products as well as samples must be considered as potentially infectious. They have to be handled with appropriate care and in strict observance of safety regulations. The rules pertaining to disposal are the same as applied to disposing hospital waste.
- Calibrators and control plasmas are made from human blood and any individual plasma involved in the procedure is HBsAg, HIV 1/2 Ab and HCV-Ab-negative (see labels on the vials). However, all human blood products should be handled as potentially infectious material.
- Stopping solution (sulphuric acid) may irritate the skin. Should acid get into your eyes, wash out immediately with water and consult a doctor.
- The reagents sometimes contain preserving agents (merthiolate). Beware of swallowing! Avoid contact with skin or mucous membranes

**LIMITATION OF THE TEST**

- Samples with high concentrations of other than anti ADAMTS-13 autoantibodies may result in weak positive or borderline results.

**REFERENCES**