THISTLE SCIENTIFIC

SERUM PROTEINS
ELECTROPHORESIS kit

For **Manual Technique**

Kit Ref.	*N° tests*	*Contents*
CSL-CASP-M	**200**	25 Cellulose Acetate Supported Membranes 7.6 x 6.2 cm (8 samples /membrane) - SP Buffer 10X 100mL - Ponceau S 100mL -Destain Solution 20X 250mL- 25 BLOTTING PAPER- 4 felted WICKS for CSL-BRD-S1 Bridge- 8 PADS (to dry the teeth of the sample applicator CSL-DROPS-S1)
CSL-CASP/4-M	**100**	25 Cellulose Acetate Supported Membranes 7.6 x 3.5 cm (4 samples /membrane) - SP Buffer 10X 100mL - Ponceau S 100mL -Destain Solution 20X 250mL- 25 BLOTTING PAPER- 4 felted WICKS for CSL-BRD-S1 Bridge- 8 PADS (to dry the teeth of the sample applicator CSL-DROPS-S1)

PRINCIPLE OF THE METHOD

Electrophoresis allows the separation of proteins in biological samples like serum, plasma and urine. Separation of serum proteins is carried out with a buffer solution at alkaline pH (pH=8.9). proteins are then fixed and stained with Ponceau S Red, while subsequent destaining with an acidic solution removes unbound stain. At the end of the process, a pattern with five basic bands will be displayed on the strip; the bands, also called 'fractions', are the proteins present in the serum and are referred as Albumin, Alpha 1, Alpha 2, Beta e Gamma, according to their mobility. Variations of their concentration and position, as the appearance of abnormal bands, have an important, qualitative diagnostic value. Densitometry of the stained bands and data elaboration by a dedicated software give a graph showing the five fractions, whose relative concentration is expressed by percentage values.

REAGENTS and MEMBRANES

Reagents and strips are IVD (In Vitro Diagnostics) certified.
Reagents must be stored between 15 and 25° C, protected from light. Kit components are stable up to the expiry date shown on the label.

REAGENT R1: Migration Buffer pH= 8,9. (10X concentrated solution)
Dilute 50 ml of R1 to the final volume of 500 ml with clean, distilled water. The diluted buffer solution is stable for 6 months stored at room temperature.
WARNING: FOR IN VITRO DIAGNOSTIC USE. HARMFUL IF SWALLOWED.
Storage and Stability: The 10X buffer is stable until the expiration date shown on the label of the bottle.
Signs of Deterioration: Discard buffer solution if it shows signs of deterioration (change of colour, presence of turbidity and/or suspension particle).

REAGENT R2: Destaining Solution, (20X concentrated solution).  **GHS07**

Prepare ready to use solution by bringing the 250 ml of R2 to the final volume of 5 litres with clean, distilled water.
Important: check that at least 1 litre of destain solution is in the tank, before starting the analysis.
Composition: Destaining solution contains Citric Acid and Orthophosphoric Acid in aqueous solution.
WARNING: CORROSIVE. FOR IN VITRO DIAGNOSTIC USE. DO NOT INGEST. HARMFUL IF SWALLOWED.
Storage and Stability: Destaining solution is stable until the expiration date shown on the label of the bottle.
Signs of Deterioration: Discard if change of color or turbidity is observed.

REAGENT R3: Ponceau S Red, 100 ml. Ready to use.  **GHS05**
Composition: Stain solution contains 0.5% (w/v) Ponceau S Red in 7,5% (w/v) Trichloroacetic acid an aqueous solution.
WARNING: CORROSIVE. FOR IN VITRO DIAGNOSTIC USE. DO NOT INGEST. HARMFUL IF SWALLOWED.
Storage and Stability: Stain solution is stable until the expiration date shown on the label of the bottle.
Signs of Deterioration: Discard if change of color is observed.

CELLULOSE ACETATE MEMBRANES, MYLAR® SUPPORTED. Ready to use.
WARNING: FOR IN VITRO DIAGNOSTIC USE
Storage and Stability: The strips are stable up to expiry date shown on the label.

Other items contained in the kit:
. Wicks for migration chamber
. Absorbent paper for sample plate

Other items required for the tests NOT included in the kit:
-Power Supply 0-300 v - Migration chamber -Sample applicator
--Box for membrane soaking in buffer solution – Bridge for membranes
-Tanks for membrane staining and destaining -Densitometre

Specimen

Fresh serum is the sample of choice. Avoid samples showing haemolysis or signs of deterioration or clots. Samples can be stored at 2-8°C for 48 hours.

ELECTROPHORETIC PROCEDURE

Buffering: soak the membrane in the buffer using the appropriate vertical container. Let the membrane for at least 5 minutes. Do not touch the acetate side with fingers.

Preparation of the migration chamber: pour the buffer solution into each of the two compartments; tilt the chamber to get the same level of buffer in both compartments.Place the two wicks in buffer.

Sample plate: load samples in the wells; avoid bubble formation during sample pipetting.

Sample application: remove the membrane from the buffer. Blot the excess of buffer. Apply samples on the membranes for 10 seconds. Make sure that the samples are completely absorbed in the membrane, then place it on the bridge with the application side down and the edge of sample application at the cathodic side of the migration chamber.

Electrophoretic migration: Plug the negative and positive cables to the power supply, cover the migration chamber and turn on the power supply.

Operative settings:

Parameter	Value	Parameter	Value
Soaking	5 min	Electrophoretic migration voltage	220 V
Staining	5 min	Electrophoretic migration time	15 min (Micro technique) 25 min (Semi-micro technique)
Destaining (3 steps using the rotating shaker).	3 min each step		

Waste Disposal

All components of the kit must be treated as hazardous or special waste and discarded accordingly.

DOMINIQUE DUTSCHER SAS