



### CH50 Eq

For *In Vitro* Diagnostic Use

The MicroVue CH50 Eq EIA measures the hemolytic complement (CH50) in human serum and allows detection of a deficiency of one or more of the complement components C1 through C9.

The binding of the C1q component of C1 to immune complexes triggers the classical complement pathway. This activation results in a cascade of enzymatic and non-enzymatic reactions, culminating in the formation of terminal complement complexes (TCC). Under standard conditions the level of TCC that can be generated in serum as a quantitative expression of the serum's classical complement activity.

The traditional method for measuring the classical complement activity is the CH50 test. This is a lytic assay, which uses antibody-sensitized sheep erythrocytes (EA) as the activator of the classical complement pathway and various dilutions of the test serum to determine the amount required to give 50% lysis. This percent hemolysis is determined spectrophotometrically. The CH50 test is an indirect measure of TCC, since the TCC themselves are directly responsible for the hemolysis that is measured.

The MicroVue CH50 Eq EIA provides a direct measure of the classical complement activity in serum by quantifying the amount of TCC generated under standard conditions. This assay uses a monoclonal antibody to a unique neoantigen to capture the TCC analyte. The CH50 Eq EIA relies on the generation of TCC of which the results are expressed in CH50 unit equivalents per milliliter.

#### Format

- ELISA
- 96-well microplate with reagents sufficient to test 40 samples in duplicate
- Sample type: Human serum
- Controls: Normal, low included

#### Assay Steps

- Prepare Reagents and Controls
- Add 86  $\mu$ L of Activator to dilution tubes
- Add 14  $\mu$ L of Controls and Specimens to dilution tubes
- Incubate 60  $\pm$ 1 minutes at 37°C

- Dilute activated controls and specimens 1:200 with Complement Specimen Diluent
- Add ~300  $\mu$ L Wash Solution
- Incubate 2 minutes at 15°C to 30°C
- Remove liquid from wells
- Pipette 100  $\mu$ L Standards, Controls and Specimens into assay wells
- Incubate 60  $\pm$ 1 minutes at 15°C to 30°C
- Wash 7 times with Wash Buffer
- Pipette 50  $\mu$ L of Conjugate
- Incubate 60  $\pm$ 1 minutes at 15°C to 30°C
- Wash 7 times with Wash Buffer
- Pipette 100  $\mu$ L of Substrate Solution

- Incubate 15  $\pm$ 1 minutes at 15°C to 30°C
- Pipette 100  $\mu$ L of Stop Solution
- Read the OD at 450 nm

#### Assay Performance

**Method:** ELISA

**Analyte:** TCC

**Specimen Volume:** 14  $\mu$ L

**Precision: (Inter-assay):** 5.4%-8.7%

**Precision: (Intra-assay):** 3.2%-4.5%

**Assay Time:** Approx. 4 hours

**Sensitivity:** 93.2%

**Specificity:** 99.4%

