PEVIVA

M65 EpiRat™ ELISA

REF 10060

Instructions for Use

For research and laboratory use only.
Not for human or diagnostic use.

M65 EpiRat™ ELISA

Instructions for Use of the M65 EpiRat™ ELISA

Contents

Explanation of Symbols Used on Labels

	Catalogue number
	Contains sufficient for <n> tests
	Batch code
	Manufacturer
	Temperature limitation
	Use by
	Consult Instructions for Use

Trademarks

M30®, Apoptosense®, M65®, EpiDeath® and PEVIVA® are registered trademarks of VLVbio AB.

Shipping and Storage

The M65 EpiRat™ ELISA is shipped in cooled conditions and should be stored at 2–8 °C. ***Note!*** Do not freeze!

Assay Description

Intended Purpose

The M65 EpiRat™ ELISA is a one-step in vitro immunoassay for the quantitative determination of soluble keratin 18 in rat serum and plasma.

Principle of the Method

The M65 EpiRat™ ELISA is a solid-phase sandwich enzyme immunoassay. Standards, controls and samples react with a solid phase capture antibody M6 directed against K18 and the HRP-(horseradish peroxidase) conjugated M5 antibody directed against a different epitope. Unbound conjugate is removed by a washing step. TMB Substrate is added. The colour development is stopped and the absorbance is read. The resulting colour is directly proportional to the concentration of the analyte.

By plotting a standard curve from known concentrations versus measured absorbance, the amount of antigen in the sample can be calculated. The concentration of the antigen is expressed as units per litre (U/L).

Materials Provided for 96 Determinations

M6 Coated Microstrips: One microplate, 12 strips with 8 wells each, 96 dry wells in total. The wells are coated with mouse monoclonal K18 antibody M6. The microplate is sealed in an aluminium bag, which contains a desiccating device. If not all the strips are used, reseal the bag and keep the desiccating device inside. ***Ready for use!***

M65 HRP Conjugate: Concentrate (24 × conc). One vial containing 0.4 mL of mouse monoclonal M5 antibody (anti-rat-K18) conjugated with horseradish peroxidase (HRP) in phosphate buffer with protein stabilizers. Preservative added. Should be diluted with M65 Conjugate Dilution Buffer. ***Note!*** Do not expose to light!

M65 Conjugate Dilution Buffer: One vial containing 11 mL of phosphate buffer with protein stabilizers for dilution of the M65 HRP Conjugate. Preservative added. Blue coloured. ***Ready for use!***

M65 Standard A– E: Standard A containing 2 mL of phosphate buffer with foetal calf serum (FCS). Standard B – E, 0.5 mL each, containing standard material in phosphate buffer with FCS. The values of Standard A– E are 0, 250, 500, 1000, 2000 U/L, respectively. Preservative added. Yellow coloured. ***Ready for use!***

Wash Tablet: One tablet for 500 mL of prepared wash solution. Dissolve the Wash Tablet in 500 mL of fresh deionised water.

TMB Substrate: One bottle containing 22 mL of TMB (3,3',5,5'-Tetramethyl- benzidine) Solution. ***Note!*** Do not expose to light! ***Ready for use!***

Stop Solution: One vial containing 7 mL of 1.0 M sulphuric acid. ***Ready for use!***

Sealing Tape: One (1) sheet.

Instructions for Use.

Certificate of Analysis.

Materials Required but not Provided

- Microplate reader (wavelength: 450 nm; linear 0–3 OD)
- Microplate shaker (oscillation: 600 rpm, orbit: 1.5–4 mm)
- 96-well microtiter plate washer or multichannel pipette (volume 250 µL)
- Vortex mixer
- Precision pipettes: 25, 50, 75 and 200 µL
- Cylinder (500 mL)
- Deionised water

Assay Protocol

Warnings and Precautions

1. M65 EpiRat™ ELISA kit is intended for Research Use Only.
2. Do not mix reagents from different kit lots.
3. All sample specimens should be regarded as contagious and handled and disposed of according to appropriate regulations.
4. Do not use samples that are contaminated.
5. The Stop Solution contains 1.0 M sulphuric acid, which will cause irritation of the skin and is harmful to the eyes. In case of contact, flush with plenty of water and seek medical advice.

Component Preparation

Dilution of M65 Conjugate

Dilute the M65 HRP Conjugate with M65 Conjugate Dilution Buffer. The M65 HRP Conjugate vial contains exactly 0.4 mL. Add 9.2 mL of the M65 Conjugate Dilution Buffer directly to the M65 Conjugate vial and mix.

Dissolving of Wash Tablet

Dissolve one Wash Tablet in 500 mL of fresh deionised water.

Storage and Shelf Life

Store reagents in their original containers at 2 – 8 °C.

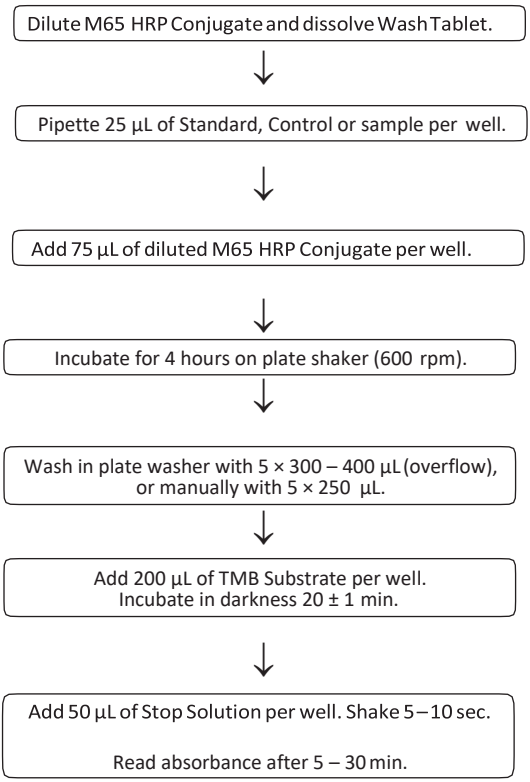
The TMB Substrate and the M65 Conjugate are sensitive to light and metal ions and should be stored in the original amber bottles at 2 – 8 °C at all times. TMB Substrate cannot be used after exposure to light.

Assay Procedure

The M65 EpiRat™ ELISA should be performed at room temperature (24 ± 3 °C).

1. Allow all reagents to reach room temperature before performing the assay. Vortex all reagents prior to use.
2. Dissolve the Wash Tablet in fresh deionised water (see "Component Preparation").
3. Dilute the M65 HRP Conjugate with M65 Conjugate Dilution Buffer (see "Component Preparation") and mix.
4. Pipette 25 µL of M65 Standard (A– E) or sample per well (duplicates are recommended).
5. Add 75 µL of the diluted M65 Conjugate solution to each well.
   Note! *Steps 4 and 5 should be performed sequentially without interruption within 20 minutes.*
6. Cover the wells with sealing tape or a microtiter plate lid.
7. Incubate on shaker for four (4) hours. Speed setting: 600 rpm.
8. Wash the plate in a plate washer five (5) times with 300 – 400 µL/well of Wash Tablet solution (overflow wash)
   or
   Wash the plate manually, discarding the incubation solution and washing the wells five (5) times with 250 µL of Wash Tablet solution. Avoid contamination between wells.
9. Add 200 µL of TMB Substrate to each well. Incubate in darkness at room temperature for 20 ± 1 minutes.
10. Add 50 µL of Stop Solution to each well. To ensure complete mixing of the TMB Substrate and the Stop Solution, shake the microplate for 5 – 10 seconds. Leave the microplate for 5 minutes before reading the absorbance.
11. Determine the absorbance at 450 nm in a microplate reader within 30 minutes and record the results.
12. Calculate the results as described in section "Calculation of Analytical Results".

Flow Chart


Dilute M65 HRP Conjugate and dissolve Wash Tablet.
Pipette 25 µL of Standard, Control or sample per well.
Add 75 µL of diluted M65 HRP Conjugate per well.
Incubate for 4 hours on plate shaker (600 rpm).
Wash in plate washer with 5 × 300 – 400 µL (overflow), or manually with 5 × 250 µL.
Add 200 µL of TMB Substrate per well. Incubate in darkness 20 ± 1 min.
Add 50 µL of Stop Solution per well. Shake 5 – 10 sec.
Read absorbance after 5 – 30 min.

Calculation of Analytical Results

The M65 EpiRat™ ELISA results are calculated using computer-assisted methods. Evaluate the values of controls and samples using a suitable program for handling ELISA-type data. Fitting algorithm: Cubic Spline. x-axis: concentration (U/L); y-axis: absorbance at 450 nm (A450).

Note! If samples have been diluted, the observed concentration must be multiplied by the dilution factor.

PEVIVA Products

M30 Apoptosense® ELISA
Prod No. 10011

M65® ELISA
Prod No. 10020

M30 CytoDeath™ ELISA
Prod No. 10900

M65 EpiDeath® ELISA
Prod No. 10040

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Date of issue: 2019-06-27