PEVIVA

M65 EpiRat™ ELISA

REF 10060

Instructions for Use

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

M65 EpiRat™ ELISA

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Instructions for Use of the M65 EpiRat™ ELISA

Contents

Explanation of Symbols Used on Labels

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

Trademarks

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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.

Summary and Explanation of the Test

Extracellular K18 can be used as a marker for epithelial cell death. During necrosis, loss of cell membrane integrity will result in the release of intracellular proteins, including K18, into the extracellular compartment. Apoptosis represents an active form of cell death that initially preserves plasma membrane integrity but which is commonly followed by secondary necrosis where intracellular components are released. The M65 EpiRat™ ELISA assay measures total soluble K18 released from dead cells (necrotic and apoptotic). Measurements from rat serum/plasma samples by the M65 EpiRat™ ELISA will therefore represent the total epithelial cell death by any cause.

The M65 EpiRat™ ELISA uses two mouse monoclonal antibodies (clone M5, Ig-G1 , and M6, IgG2a) specific for conventional epitopes of K18. The M5 antibody detects rat K18, but does not react to human or mouse K18.

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

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

M65 EpiRat 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'-Tetramethylbenzidine) Solution. ***Note!*** Do not expose to light! *Ready for use!*

Stop Solution: One vial containing 8 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. Do not use samples that are contaminated.
4. 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.
5. Safety Data Sheets (SDS) are available on www.vlvbio.com or by request.

Collection and Preparation of Samples

The sample volume should be sufficient for measuring each sample in duplicate (test volume 2 x 25 µL).

Store samples at 2-8°C up to 4 hours. For longer periods store samples frozen at -20 °C or lower. Samples can be freeze-thawed without loss of activity but it is recommended that repeated freeze-thawing should be avoided.

Note! The assay has not been tested on rat cell lines and thus the functionality can not be guaranteed. Please consult www.vlvbio.com for further information on the performance of the M65 EpiRat™ ELISA.

Component Preparation

Dilution of M65 EpiRat Conjugate

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

Dissolving of Wash Tablet

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

Dilution of Samples

Samples higher than Standard E should be diluted with rat blood serum or Standard A (0 U/L). Since dilution in the assay is linear, the original concentration is calculated by multiplying the measured concentration by the dilution factor. In case blood serum was used as sample diluent, its concentration (U/L) must be accounted for.

Storage and Shelf Life After First Opening

If the entire kit is not used, ,store reagents in their original containers at 2 – 8 °C. If not all strips are used, reseal the microstrips bag. Remember to include the desiccating device.

The TMB Substrate and the M65 EpiRat Conjugate are sensitive to light and metal ions and should be stored in the original amber bottles at 2 – 8 °C at all times between uses. If a new container is used it has to be protected from light! TMB Substrate cannot be used after exposure to light.

Note! Only dilute the amount of M65 EpiRat Conjugate that you will use at the occasion. Store the undiluted M65 EpiRat Conjugate in the original amber bottle at 2-8°C. Do not expose to light.

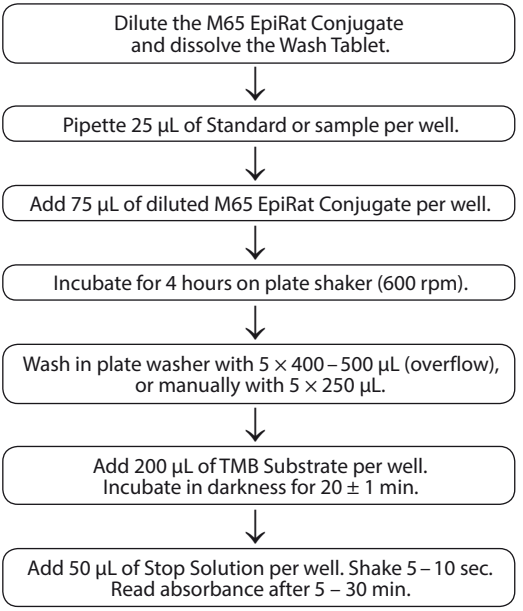
The Wash Tablet solution is stable for 5 weeks when stored at 2 – 8 °C.

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 EpiRat Conjugate with M65 EpRat Conjugate Dilution Buffer (see "Component Preparation") and mix.
4. Pipette 25 µL of M65 EpiRat Standard (A – E) or sample per well (duplicates are recommended).
5. Add 75 µL of the diluted M65 EpiRat 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 400 – 500 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 the M65 EpiRat Conjugate and dissolve the Wash Tablet.
Pipette 25 µL of Standard or sample per well.
Add 75 µL of diluted M65 EpiRat Conjugate per well.
Incubate for 4 hours on plate shaker (600 rpm).
Wash in plate washer with 5 × 400 – 500 µL (overflow), or manually with 5 × 250 µL.
Add 200 µL of TMB Substrate per well. Incubate in darkness for 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 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: con- centration (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, and in case rat blood serum/plasma was used as sample diluent, its M65 concentration (U/L) must be accounted for. The concentration of undiluted sample is calculated by following the mathematical formula:

$c_{neat} = \{c_{obtained} - [(1 - \text{dilution factor}) \times c_{diluent}]\} \div \text{dilution factor}$

c_{neat} = the neat concentration of the sample in U/L

$c_{obtained}$ =the obtained concentration of the diluted sample in U/L

$c_{diluent}$ =the concentration of the diluent in U/L

Example:

A sample exceeding the highest kit standard (M65 Standard E) is diluted 1:50 with a rat blood serum sample. The diluted sample and the blood donor serum are measured in the assay. The obtained concentrations of the diluted sample and the blood donor serum are 250 U/L and 100 U/L respectively.

dilution factor = 1:50 = 0.02

$c_{obtained}$ = 250 U/L

$c_{diluent}$ = 100 U/L

$c_{neat} = \{250 - [(1 - 0.02) \times 100]\} \div 0.02 = 7\,600$ U/L

Thus, the concentration of the undiluted high sample is 7 600 U/L.

Assay Performance

Performance Characteristics

Measuring range: The measuring range is 0 – 2 000 U/L;

Sensitivity: Limit of Detection (LOD) is 50 U/L (calculated as standard A + 3 standard deviations); Lower Limit of Quantification (LLOQ) is 98 U/L.

Reproducibility: Within assay (WA % CV) variation is < 7 %, between assay (BA % CV) variation is < 10 % and total variation < 10 % for samples over LLOQ.

Linearity/Dilution: Recovery within 80-120 % for dilutions in rat serum or Standard A.

Warranty

The performance data presented here were obtained using the procedure indicated. Any change or modification in this procedure as recommended by the manufacturer may affect the results. In such event, the manufacturer disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and the fitness for use. The manufacturer and its authorized distributors, in such event, shall not be liable for damages indirect or consequential.

VLVbio
VLVbio AB, Hästholmsvägen 32, 131 30 Nacka, Sweden
Phone: +46 8 122 053 00 • www.vlvbio.com • info@vlvbio.com

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