QUIDEL

MicroVue™ Complement

Pan-Specific C3 REAGENT KIT

A set of reagents used to detect the depletion of Complement protein C3 in various animal species.

PROCEDURE SUMMARY

NOTE: Read full instructions for use prior to performing testing.

Reagents Preparation

- ☐ Thirty (30) minutes prior to use, rehydrate C3 Complement Matrix with 0.9 mL deionized water, mix and place in ice bath.
- ☐ Rehydrate Positive Control with 200 µL deionized water, mix and place in ice bath.
- ☐ Vortex before use.

Assay Procedure

Place empty tube in an ice bath

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Add **20 µL** animal samples or **20 µL** Positive Control (or **20 µL** Specimen Diluent for blank determination)

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Add **20 µL** C3 Complement Matrix

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Add **100 µL** Specimen Diluent

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Add **10 µL** C3 Converter Reagent; Vortex

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Incubate **1 hour** at 37˚C (water bath)

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Add **10 µL** Stop Solution, vortex. Dilute serum samples 1:2.5 with Specimen Diluent, dilute plasma samples 1:10 with Specimen Diluent and store in an ice bath until assayed or freeze samples at –70˚C or below

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Measure samples in the MicroVue SC5b-9 Plus EIA according to the kit instruction

INTENDED USE

The MicroVue Pan-Specific C3 Reagent Kit in conjunction with the MicroVue SC5b-9 Plus EIA, detects the depletion of Complement protein C3 in various animal species.

SUMMARY AND EXPLANATION

The complement system (C-system) is a protein cascade of the immune system consisting of greater than 30 plasma and cell membrane proteins. It provides a first line of defense against microbial attacks against the body, orchestrating their clearance and initiation of long-term immunity. As revealed recently, the C-system also has important activities in some non-immune physiological processes, such as conception and tissue regeneration. Besides its essential role in maintaining health, the importance of the C-system in medicine lies in the fact that a large number of acute and chronic diseases are associated with abnormalities in complement function. Accordingly, quantitative analysis of different complement proteins and their activation by-products are of great practical importance in experimental and clinical medicine. There are numerous immunological methods for complement testing, including ELISA-based kits measuring complement cleavage products as markers of complement activation. However, most of the commercially available ELISAs are human specific, leaving an expanding need for animal testing of complement activation largely unmet.

PRINCIPLE OF THE PROCEDURE

The MicroVue Pan-Specific C3 Reagent Kit represents a novel approach to fill the gap of animal-specific C-ELISAs. The C3 Complement Matrix (CCM) and the C3 Converter Reagent (CCR) in the kit convert the activity of C3 in the animal specimen to human SC5b-9 that is detectable with the MicroVue SC5b-9 Plus EIA kit. Thus the method allows sensitive and quantitative measurement of C3 in animal blood, plasma or serum, which, to the extent C3 had been consumed prior to the assay, also provides a measure of prior complement activation. The method expands the methodical arsenal of complement analysis in animals, enabling, among many applications, preclinical immune toxicology testing of complement mediated (pseudoallergic) adverse drug effects.[1, 2, 3, 4]

The PS-C3 method is a three-step procedure. In the first step animals are treated with, or anima sera or plasma samples are incubated with, the test drugs, agents, or devices to explore possible complement activation. Samples are collected at appropriate times for the second step: conversion of animal C3 to human SC5b-9. Unless this step is immediately (within hours) performed, samples should be frozen and stored at –70°C or below, or according to the previously established laboratory protocol for serum or plasma sample handling for complement testing. C3 conversion is performed as specified in the MicroVue Pan-Specific C3 Reagent Kit. In the third step SC5b-9 is measured using the human SC5b-9 Plus EIA kit.

REAGENTS AND MATERIALS PROVIDED

1. C3 Complement Matrix Part 1254400 Lyophilized powder
   Reconstitute powder with 0.9 mL deionized water and mix thoroughly. Place in ice bath or prepare aliquots and store unused C3 Complement Matrix immediately at –70°C or below
2. Specimen Diluent Part 1254700 10 mL
   Store at 2°C to 8°C. Ready to Use.
3. C3 Converter Reagent Part 1254500 0.5 mL suspension
   Vortex the C3 Converter Reagent immediately before and during use, approximately every 10 samples. Store at 4°C. Ready to Use.

❹ Stop Solution Part 1254600 0.5 mL 0.2M EDTA.
Store at 2°C to 8°C. Ready to use.

❺ Positive Control Part 1267200 Lyophilized Powder.
Reconstitute powder with 200 µL deionized water and mix thoroughly. Place in ice bath for the assay or prepare aliquots and store unused Positive Control immediately at –70°C or below.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Timer (60-minute range)
2. Clean, unused test tubes and racks and microassay plates
3. Deionized or distilled water
4. Water bath capable of 37°C
5. Micropipettes and pipette tips
6. MicroVue SC5b-9 Plus EIA kit if a measured result is desired

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. Treat specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any patient samples.
3. Dilution adjustments may be required depending on the Complement status in the test animal. Ensure that the appropriate dilution factor is used to generate actual analyte concentrations.
4. Dispose of containers and unused contents in accordance with Federal, State and Local regulations.
5. Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
6. Store assay reagents as indicated.
7. ProClin 300 is used as a preservative. Incidental contact with or ingestion of buffers or reagents containing ProClin can cause irritation to the skin, eyes or mouth. Use good laboratory practices to reduce exposure. Seek medical attention if symptoms are experienced.
8. Wear Nitrile or Latex gloves and protective eyewear when handling the chemical and/or biological components of this kit.
9. Proper collection and storage of test specimens are essential for accurate results (see Specimen Collection and Storage).
10. Avoid microbial or cross-contamination of specimens or reagents.
11. Decontaminate all specimens, reagents, and materials by soaking for a minimum of 30 minutes in a 1:10 solution of household bleach (sodium hypochlorite) or autoclave at 121°C for 30 minutes at 15 psi.
12. Using incubation times and temperatures other than those indicated in the Procedure section may give erroneous results.
13. Heat-inactivated specimens may yield erroneous results.
14. Hyperlipemic or contaminated specimens may give erroneous results.

REAGENT PREPARATION

Reconstitute C3 Complement Matrix with 0.9 mL deionized water and mix thoroughly. Place in ice bath for the assay or prepare aliquots and store unused C3 Complement Matrix immediately at –70°C or below.

Reconstitute Positive Control with 200 µL deionized water and mix thoroughly. Place in ice bath for the assay or prepare aliquots and store unused Positive Control immediately at –70°C or below.

After removing the needed reagents and materials, return the unused items to their appropriate storage temperatures (see Storage).

STORAGE

Store the unopened kit at 2°C to 8°C.

Upon reconstitution, the Positive Control and C3 Complement Matrix should be stored on ice until used or aliquoted and frozen at –70°C or below for future use.

SPECIMEN COLLECTION AND STORAGE

1. Sample type:
   - Serum
   - 10-15 IU/mL heparin plasma or 50 µg/mL hirudin plasma for anticoagulation
2. **EDTA or citrate plasma cannot be used.**
3. If testing is not to be performed immediately, aliquot samples into appropriate volumes and store frozen at –70°C or below.
4. For previously frozen specimens, thaw samples rapidly in a 37°C water bath until just thawed. Immediately place samples in an ice bath until use.

ASSAY PROCEDURE

1. 30 minutes prior to use, reconstitute C3 Complement Matrix with 0.9 mL of deionized water and store on ice until use.
2. Reconstitute Positive Control with 200 µL of deionized water and store in ice until use.
3. Label appropriate test tubes with corresponding sample data and place in ice bath.
4. Take blood sample from treated (and control) animals and prepare plasma; or incubate test drug with animal serum or plasma.
5. Pipette 20 µL of animal sample or positive control into corresponding test tube (or specimen diluent for a blank determination).
6. Add 20 µL of reconstituted C3 Complement Matrix.
7. Add 100 µL of Specimen Diluent.
8. Add 10 µL of C3 Converter Reagent.
9. Vortex.
10. Incubate for 1 hour in a 37°C water bath.
11. Add 10 µL Stop Solution.
12. Vortex.
13. Further dilute serum reaction mixture 1:2.5 with Specimen Diluent (i.e. 20 µL reaction mixture + 30 µL Specimen Diluent), or dilute plasma reaction mixture 1:10 with Specimen Diluent (i.e. 10 µL reaction mixture + 90 µL Specimen Diluent).
14. Store remaining reaction mixture and diluted sample in an ice bath until assayed or freeze at –70°C or below.
15. Measure samples using MicroVue SC5b-9 Plus EIA kit according to the Package Insert instructions.

EXPERIMENTAL SETUP EXAMPLE

In Vivo

1. Apply drug or device to test animal (i.e., the concentration which will be applied for treatment later in humans + 10 times more).

2. Animal testing always shows significant individual variation in response; therefore, it is generally recommended to test 6-10 animals. Ten (10) or more individual animals should be evaluated when using the rat model.
3. Blood Collection:
   a) Collect samples from treated and non-treated animals. Suggested sampling pattern at time 0 and after application at 2, 5, 10, 15, 30, 60 and 120 minutes.
      i) It is suggested that the Time 0 sample is evaluated both with the Complement Activator and without the Complement Activator as an additional experimental control.
   b) Immediately prepare plasma or serum and assay. Alternatively, store samples at –70°C for testing at a later time.

2) Apply the MicroVue Pan-Specific C3 assay procedure for all samples, followed by measurement using the MicroVue SC5b-9 Plus EIA.

In Vitro

1) Add different concentrations of drug or material of device to animal serum. Store one sample untreated as a negative control and include a positive control by spiking a sample with HAGG or Zymosan. CVF is not recommended for use as an *in vitro* activator as CVF can bind to fragment Bb and form a C5 convertase. The C5 convertase can generate elevated levels of TCC in the absence of animal C3)[6].
   a) It is suggested that the Time 0 sample is evaluated both with the Complement Activator and without the Complement Activator as an additional experimental control.
2) Incubate at 37°C and collect samples after 15, 30, 60 and 120 minutes. Immediately place samples on ice.
3) Apply the MicroVue Pan-Specific C3 assay procedure for all samples, followed by measurement using the MicroVue SC5b-9 Plus EIA.

QUALITY CONTROL

Good laboratory practice recommends use of controls to ensure that the assay is performing properly. Each MicroVue Pan-Specific C3 Reagent Kit contains a Positive Control for this purpose. Each laboratory should establish its own parameters for acceptable assay limits. If the control values are NOT within your laboratory's acceptance limits, the assay results should be considered questionable, and the samples should be repeated.

ASSISTANCE

To place an order or for technical support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at 740.592.9820. For e-mail support, contact custserv@quidel.com or technicalsupport@quidel.com.

For services outside the U.S., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website quidel.com.

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REF 20261 – MicroVue Pan-Specific C3 Reagent Kit

RUO

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REF Catalogue number	CE mark of conformity
LOT Batch code	Use by
Manufacturer	Temperature limitation
Intended use	Consult e-labeling instructions for use
WARNING: Harmful if swallowed (oral)	Biological risks
RUO For research use only	Σ 96 Contains sufficient for 96 determinations
CONT Contents/Contains	CONTROL Control