mdbioproducts.

MonELISA® Series
Mouse IgG2 anti-Collagen Type II ELISA

Catalog Number M036081

For the quantitative determination of Anti-Collagen Type II IgG2b antibodies in mouse serum samples.

For research use only.
This product insert must be read in its entirety before use.

PRINCIPLE OF THE ASSAY

The MonELISA® Anti-Collagen Type II ELISA is a colorimetric direct immunoassay utilizing collagen bound to the surfaceof microwells and a biotinylated secondary antibody to mouse IgG2B as the detection antibody. CollagenType II is widely used for inducing disease in the collagen-induced arthritis (CIA) model. The serum antibod- ies in CIA mice are highly speci!c to the Type II Collagen used for immunization. Those antibodies bind tothe immobilized collagen. The biotinylated secondary antibody binds to serum antibodies to Collagen TypeII. Streptavidin-Peroxidase in the presence of an enzyme substrate quanti!es the antibodies bound. Colordevelopment is directly proportional to the antibody concentration in the sample.

KIT COMPONENTS

- Anti-Collagen Type II Microplate The plate contains 12 x 8 strips coated with bovine collagen type II.
- Mouse anti-Collagen Type II Standard 1 vial (250 ng/mL) of mouse anti-Collagen Type II in a
- protein buffer.
- Detection Antibody Concentrate 1 vial of a 100-fold concentrated biotinylated
- anti-mouse IgG2B antibody in a stabilizing buffer.
- Streptavidin-HRP Concentrate 1 vial of 100-fold concentrated Streptavidin-HRP in a stabilizing buffer.
- Plate Sealer – 2 adhesive strips.

THESE MONELISA® ASSAY COMPONENTS ARE TO BE USED WITH 5-PLATE ACCESSORY KIT CATALOG NUMBER M308080.

SUPPLIES REQUIRED BUT NOT PROVIDED

- Pipettes or pipetting equipment with disposable polypropylene tips
- Glass measuring cylinders
- Distilled or deionized water
- Horizontal orbital microplate reader
- Squirt bottle or automated microplate washer
- Microplate reader capable of measuring at 450 nm

PRECAUTIONS

Stop Solution consists of diluted sulfuric acid. Wear eye, hand, face, and clothing protection when using these materials. Avoid contact with skin and eyes. In case of contact wash immediately with water. All chemicals should be considered as being potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice.

The Assay Diluent contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

- For research use only. Not for internal or external use in humans or animals.
- This kit contains no material of human origin.
- For the handling of blood, (serum), we recommend that precautions should be observed.
- Please refer to HHS Publication no. (CDC) 88-8395 or corresponding local/ national guidelines on laboratory safety procedures.

CRITICAL PARAMETERS

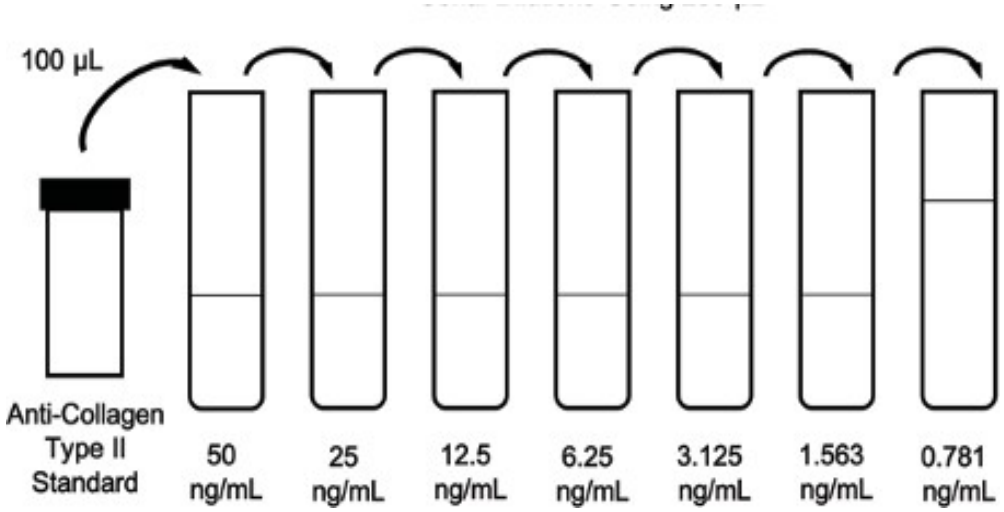
- Allow samples and all reagents to equilibrate to room temperature (20 - 30 °C) prior to performing the assay. This is especially a prerequisite for the TMB Substrate.
- It is absolutely important that all wells are washed thoroughly and uniformly. When washing is done by hand, use a squeeze bottle and ensure that all wells are completely filled and emptied at each step.
- Use only reagents from the same lot for each assay. This is especially important when running more than one plate per sample group.
- A separate standard curve must be run on each plate.
- Mix all reagents thoroughly prior to use but avoid foaming!
- Keep the wells sealed with the foil except when adding reagents and during reading.
- Any variation in the protocol can cause variation in binding!
- The kit should not be used beyond the expiration date on the kit label.
- The values obtained by the samples should be within the standard range. If this is not the case, dilute the sample and repeat the assay.
- We take great care to ensure that this product is suitable for all validated sample types, as designated in this manual. Other sample types may be tested and validated by the user.

SAMPLE COLLECTION

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C.

SAMPLE PREPARATION

Standards – Label 7 standard tubes as shown below. Pipette 400 µL Assay Diluent into the 50 ng/mL standard tube and 250 µL Assay Diluent into the remaining tubes. Use the 250 ng/mL standard to produce a 2-fold dilution series (see below). The 50 ng/mL standard serves as the high standard and the Assay Diluent serves as the (0 ng/mL) standard.



ASSAY PROTOCOL

Read the entire protocol before beginning the assay. It is recommended that all standards and samples be assayed in duplicate.

1. Prepare all reagents and samples as described in the previous sections.
2. Remove any excess microplate strips from the plate frame and return them to the foil pouch containing the desiccant pack.
3. Add 100 µL of Standard or diluted sample in duplicate to each well. Cover with the plate sealer provided and incubate for 1 hour at room temperature.
4. Aspirate and wash the wells 3 times with 200 µL per well of Wash Buffer (1X). Take care that all wells are filled and emptied at each wash. Blot the plate on paper towels to remove residual fluid from the plate.
5. Add 100 µl Conjugate (1X) to each well. Cover the plate with the plate sealer provided and incubate for 1 hour at room temperature.
6. Aspirate and wash the wells 3 times with 200 µL per well of Wash Buffer (1X). Take care that all wells are filled and emptied at each wash. Blot the plate on paper towels to remove residual fluid from the plate.
7. Add 100 µL of diluted Streptavidin-HRP to each well. Incubate for 30 minutes at room temperature. Protect from light.
8. Aspirate and wash the wells 3 times with 200 µL per well of Wash Buffer (1X). Take care that all wells are filled and emptied at each wash. Blot the plate on paper towels to remove residual fluid from the plate.
9. Add 100 µL Substrate to each well and incubate for 15 minutes at room temperature. Protect from light.
10. Stop the reaction by adding 100 µL of Stop Solution to each well. Gently tap the sides of the plate to ensure thorough mixing.
11. Read the plate at 450 nm.

SUMMARY

Prepare reagents and samples as previously described.

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Pipette 100 µL Standard or diluted sample in duplicate to each well.

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Cover with plate sealer and incubate 1 hr. at room temperature.

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Aspirate and wash 6 times.

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Add 50 µL of Conjugate (1X) to each well.

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Cover with plate sealer and incubate 1 hr. at room temperature.

↓

Aspirate and wash 6 times.

↓

Add 100 µL of diluted Streptavidin-HRP to each well. Incubate 30 min. at room temperature. Protect from light.

↓

Aspirate and wash 6 times.

↓

Add 100 µL of Substrate to each well. Incubate 15 min. at room temperature. Protect from light.

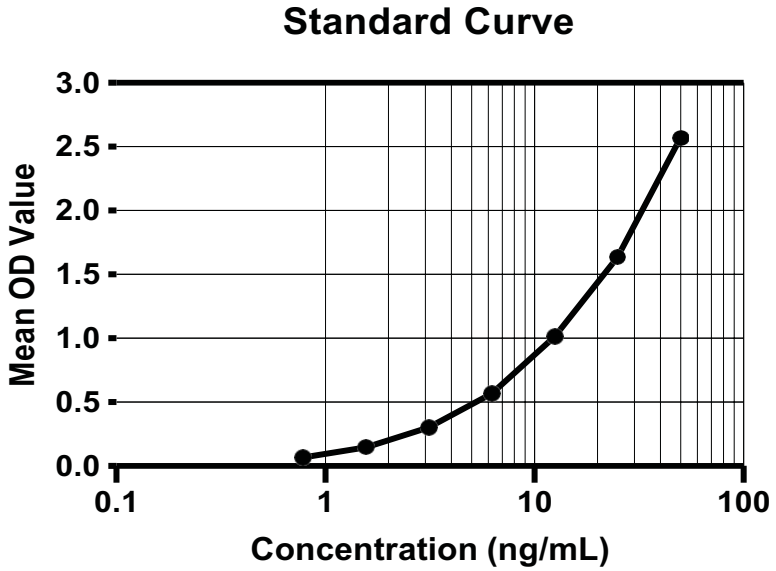
↓

Add 100 µL of Stop Solution to each well. Read at 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve it. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best it curve through the points on the graph. The data may be linearized by plotting the log of the collagen type II concentrations versus the log of the O.D. and the best it line can be determined by regression analysis. This procedure will produce an adequate but less precise it of the data.

This standard curve is provided for demonstration only. A standard curve should be generated with each set of samples assayed.



SENSITIVITY

Sensitivity is de!ned as the minimal detectable dose determined by adding two standard deviations of the mean optical density value for twenty replicates of the zero standard and calculating the corresponding concentration. The sensitivity of the MonELISA® Anti-Collagen Type II ELISA is typically less than 0.75 ng/mL.

REPRODUCIBILITY

	Intra-assay Precision			Inter-assay Precision		
Control	1	2	3	1	2	3
Mean (ng/mL)	2.5	9.9	30.3	2.5	10	29.8
Standard Deviation	0.16	0.49	1.5	0.17	0.55	1.57
CV (%)	6.6	5	4.9	6.7	5.5	5.3

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