cellsciences.com

# Mouse IgG Easy Quantification Kit

**Catalog No.:** IS003A **Quantity:** 1 Plate (1 x 96 tests) **Lot No.:** TBD **Exp. Date:** TBD
**Catalog No.:** IS003B **Quantity:** 10 Plates (10 x 96 tests) **Lot No.:** TBD **Exp. Date:** TBD

NOTE: this is a sample protocol which is subject to variation by Lot Number. Refer to the protocol inserted in your package for the current lot number specifications and expiration date or contact our technical support at tech@cellsciences.com

The Mouse IgG Easy Quantification Kit provides a rapid and easy method (one antibody step ELISA) for the quantitative determination of mouse IgG in cell culture supernatant and ascitic fluid. The kit includes ready-to-use reagents necessary to analyze up to 90 samples in 30 minutes. Buffer solutions are color coded to simplify pipetting steps.

**Principle of the Assay:**
The method employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific to mouse IgG (H+L) is pre-coated onto the microwells. Samples and standards are pipetted into microwells, and mouse IgG present in the sample is bound by the capture antibody. Next, a HRP (horseradish peroxidase) conjugated anti-mouse IgG (H+L) antibody is pipetted and incubated simultaneously with samples. After washing the microwells in order to remove any non-specific binding, the ready to use substrate solution (TMB) is added to microwells and color develops proportionally to the amount of mouse IgG in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

**Specificity:**
The method enables the detection of all IgG (IgG3 quantification requires a specific standard curve). Cross reactions (determined by ELISA) are < 1% for Human IgG, < 1.5% for Cow IgG, < 2% for Goat IgG, < 5 % for Swine IgG and <15 % for Guinea Pig and Rat IgG.
The cross reaction with human serum and fetal calf serum is typically below 0.2%.

**Sensitivity:**
The detection range is from 20 ng/mL to 1900 ng/mL.
The detection threshold is 6 ng/mL.

**Kit Contents (for 1 x 96 tests):**

| Item | Description | Quantity |
|---|---|---|
| IS003-P | Pre-coated microplates: 96 microwells coated with anti-mouse IgG (H+L) polyclonal antibodies | 6 strips of 16 wells (2 wells x 8 wells) |
| IS003-A | Mouse IgG standards (Blue solution)<br>Concentrations:<br>0 – 0.02 – 0.1 – 0.2 – 0.75 – 1.9 µg/mL | 6 x 300 µL |
| IS003-B | Sample Diluent (PBS pH7.4, 1% BSA, 0.1% Tween 20) (Blue solution) | 30 mL |
| IS003-C | Detection antibody: Peroxidase conjugated anti-mouse IgG (H+L) polyclonal antibody (Red solution) | 12 mL |
| IS003-D | Substrate solution (TMB) | 12 mL |

**Cell Sciences**®
65 Parker Street
Unit 11
Newburyport, MA 01950

Toll Free: 888 769-1246
Phone: 978 572-1070
Fax: 978 992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

| | | |
|---|---|---|
| IS003-E | Stop solution (2M HCl) | 12 mL |

*All the kit components are ready-to-use.*

**Cell Sciences**®
65 Parker Street
Unit 11
Newburyport, MA 01950

Toll Free: 888 769-1246
Phone: 978 572-1070
Fax: 978 992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

**Storage and Stability:**
All kit components are stable for 12 months when stored at 2-8 °C. **Do not freeze.** After opening, reagents must be handled with care to avoid contamination and should be used within 2 months.

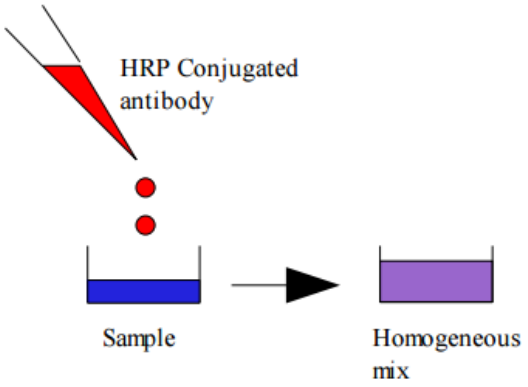
**Other Reagents and Supplies Required:**

- Pipettes and tips (20-200 µL)
- ELISA plate washer (recommended)
- Microplate reader for absorbance measurements at 450 nm and 620 nm
- Wash solution: $H_2O$, 0.05% Tween 20
  Note: Other wash solutions may be used, but they have to be tested with the method.

**Sample Preparation and Storage:**
If necessary, samples may be stored at -20 °C prior to performance of the assay. Dilute the samples in the sample diluent (Blue).
Recommended dilution factors are indicated in the following table:

| Samples | Recommended Dilutions |
|---|---|
| Cell culture supernatant | 1:100 |
| Miniperm, CELLine supernatant | 1:1,000 |
| Ascitic fluid | 1:010,000 |

**Assay procedure:**
All steps must be performed at room temperature (RT). Bring all the reagents to room temperature 30 min before use.

| | |
|---|---|
| Step 1 | Perform the dilution of each sample in diluent buffer. Serial dilutions may be necessary as previously recommended. |
| Step 2 | Add 20 µl of samples or standards per microwell. |
| Step 3 | Immediately pipette in the same order 100 µL of peroxidase conjugated anti-mouse IgG (Red solution). Mix gently until obtaining a homogeneous purple color. Incubate the microwell for 15 minutes at RT.   |
| Step 4 | After incubation, remove the solution and wash the microwells three times each with 300 µL of the wash solution. An automatic plate washer is recommended. |
| Step 5 | Pipette 100 µL of TMB substrate in each well. Incubate for 10 minutes at room temperature. |
| Step 6 | Stop the reaction by pipetting 100 µL of STOP solution in the same order as for the TMB distribution. |
| Step 7 | Read the absorbance at 450 nm and 620 nm with a microplate reader. |

**Cell Sciences**®
65 Parker Street
Unit 11
Newburyport, MA 01950

Toll Free: 888 769-1246
Phone: 978 572-1070
Fax: 978 992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

**Calculation of Results:**

***Validation of the assay:*** The mean absorbance of the 0 ng/mL standard should be below 0.1 units. Maximal absorbance (1900 ng/ml standard) should be around 1.6 to 2.2 units, depending of the operating temperature.

***Standard curve:*** Plot the average value (OD450 minus OD620) of each standard on the Y axis against their corresponding concentration on the X axis. Software able to generate a cubic spline curve-fit is recommended. The mouse IgG concentration in the sample can be calculated by interpolation between standard points on the curve.

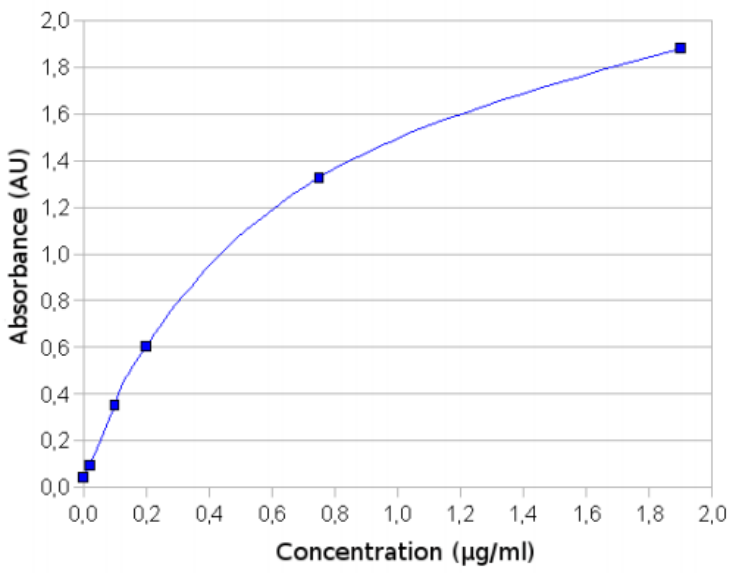
*Note:* It is recommended to repeat the assay at a different dilution factor in the following cases:

- The sample absorbance value is below the first standard.
- The absorbance value is equivalent or higher than the 1900 ng/mL standard.

***Hook effect:*** A hook effect may be observed at IgG concentrations above 5000 ng/mL. In this case, serial dilution of the sample is recommended.

**Typical Data:**

This standard curve is shown as an example only. A new standard curve should be performed for each series of samples to be tested.




**Cell Sciences**®
65 Parker Street
Unit 11
Newburyport, MA 01950

Toll Free: 888 769-1246
Phone: 978 572-1070
Fax: 978 992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

**Precision:**

*Intra-assay precision:*

| Sample | Dilution | Mean concentration (µg/ml) | SD (%) | Number of measures |
|---|---|---|---|---|
| Supernatant A | 1:100 | 10.63 | 5.33 | 9 |
| Supernatant B | 1:100 | 11.56 | 7.47 | 9 |
| Supernatant C | 1:100 | 22.61 | 8.48 | 9 |
| Supernatant D | 1:100 | 28.88 | 10.03 | 9 |
| Supernatant E | 1:100 | 66.82 | 8.39 | 9 |
| Supernatant F | 1:100 | 75.92 | 9.9 | 9 |
| Supernatant G | 1:100 | 102.47 | 10.38 | 9 |

*Inter-assay precision:*

| Sample | Dilution | SD (%) | Number of measures |
|---|---|---|---|
| Supernatant H | 1:250 | 3.45 | 30 |
| Supernatant H | 1:500 | 2.99 | 30 |
| Supernatant H | 1:1000 | 4.97 | 30 |

**NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.**