QuickQuant™

Mouse IgG Quantification Kit

Catalog #: QQNT-030, Lateral Flow Assay

Application Background

Production of mouse monoclonal antibodies (mAb) using in-vitro methods is reliable, reproducible and cost-effective. Monitoring and adjusting selected metabolic parameters during production may ensure optimal antibody yields. The concentration of mAb produced from hybridoma cells is extremely variable. Static cell cultures will produce concentration ranging from < 10 µg/mL (poor producer) to > 60 µg/mL. Other processes, such as perfusion bioreactors and hollow-fiber systems, can yield mouse IgG in excess of 4 mg/mL.

QuickQuant™ lateral flow test strips allow semi-quantitative monitoring of IgG antibody production. They provide a base-line standard for in-vitro methods, allowing the researcher/ manufacturer a rapid and constant monitoring of antibody production yields in less than fifteen minutes.

Intended Use

The BioAssay Works® QuickQuant™ Mouse IgG Lateral Flow Quantification Kit is intended for determination of mouse IgG_1, IgG_{2a}, IgG_{2b}, and IgG_3 immunoglobulin concentration in cell culture supernatant fluids. The strips provide rapid test results (< 15 minutes) to monitor semi-quantitative mAb concentration during in-process analysis.

Test Principle

BioAssay Works® QuickQuant™ Mouse IgG Lateral Flow test strips are placed in test tubes containing diluted archive samples or test samples. In the presence of mouse IgG, the gold-conjugate (anti-mouse IgG) located above the test sample pad interacts with the migrating IgG. The complex travels through the nitrocellulose membrane and precipitates on an anti-mouse IgG capture line immobilized on the membrane, forming a red line that can be read in less than fifteen minutes.

The assay provides reliable results with mouse IgG that has been diluted to a concentration ranging from 10 ng/mL to 100 ng/mL.

QuickQuant™ lateral flow tests provide semi-quantitative measurement of mouse IgG concentration.

It is recommended to use an Archive Sample with each new test sample.

An example of QuickQuant™ results is displayed in Figure 1. Actual results will vary based on specific cell culture conditions.

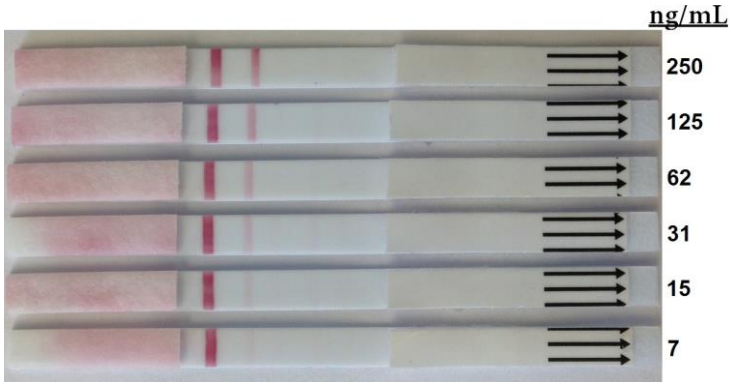

Figure 1: Serial dilutions of Mouse IgG Subclass Standard.

Materials Provided

- Mouse IgG Subclass Standard, Cat. # QQG1S-300. One vial of mouse monoclonal IgG_1 antibody (0.3 mL, 0.5 µg/mL) in phosphate buffered saline containing a protein stabilizer and preservative.
- QuickQuant™ Lateral Flow Test Strips, Cat. # QQNT-S-030. One tube containing 30 lateral flow test strips with desiccant.
- Sample Diluent Buffer, Cat. # SDB-025. One bottle containing 25 mL of SDB buffer.

Materials Required But Not Provided

- Laboratory quality distilled/deionized water.
- Microtubes or 96 well microtiter plates (low protein binding).
- Borosilicate glass test tubes, 12 x 75mm.
- Archive Samples – see Archive Sample Preparation (Page 2).

Storage and Handling

Store QuickQuant™ lateral flow test strips in tightly sealed desiccant tubes at room temperature, 20-25°C. **Do not open test strip tubes until you are ready to perform the test — minimize strip exposure to the environment.** Store the Sample Diluent Buffer (SDB-025) and Mouse IgG Subclass Standard (QQG1S-300) at 2-8°C. If the entire kit is stored at 2-8°C, allow the test strips (QQNT-S-030) to come to room temperature before opening the sealed container.

Warnings and Precautions

- For research use only.
- Do not use beyond the expiration date.
- Do not mix components from different kit lots.
- Best results are achieved by following the protocol described below, using cGLP guidelines.

Fluid Collection and Preparation

Cell culture supernatant fluid samples should be centrifuged to remove cellular debris. A preservative, such as 0.02% sodium azide, may be added to prevent microbial growth. Samples with preservative may be stored at 2-8°C for several months. For long-term or unpreserved storage, samples should be stored frozen, ≤ -20°C.

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Assay Procedure

NOTE: Allow reagents to come to room temperature before use.

I – **Primary Sample Dilution**

A primary dilution is required for all test and archive samples. A 1/100 dilution (2 µL of sample in 198 µL of Sample Diluent Buffer) of each sample should be performed in a 96-well microtiter plate or microtubes. Each sample will require one (1) well/tube. This Primary Sample Dilution is sufficient for static cell cultures (expected IgG concentrations from 10-60 µg/mL).

II – **Secondary Sample Dilution**

In-vitro production methods where the expected IgG concentration is > 100 µg/mL require a Secondary Sample Dilution. The secondary dilution may be flexible to accommodate variation in production efficiency.

Perform the Secondary Sample Dilution as detailed in Table A:

Table A: Secondary Sample Dilutions

Expected Concentration	Secondary Dilution	Volume of Primary Dilution / Volume of Sample Diluent Buffer
0.1 – 0.25 mg/mL	1/5	20 µL / 80 µL
0.25 – 0.5 mg/mL	1/10	10 µL / 90 µL
0.5 – 1.0 mg/mL	1/20	5 µL / 95 µL
1.0 – 2.0 mg/mL	1/40	2.5 µL / 97.5 µL

Testing Procedure

Following Primary and Secondary Sample Dilutions:

1. Keep kit components at room temperature before use.
2. Properly label all reaction tubes.
3. Perform a 1/10 dilution of the Archive sample and/or supplied Mouse IgG Subclass Standard in a test tube (180 µL of Sample Diluent Buffer and 20 µL of the Archive sample or Mouse IgG Subclass Standard).
4. Perform a 1/10 dilution of each sample in separate test tubes (180 µL of Sample Diluent Buffer and 20 µL of each previously diluted sample).
5. Remove the required number of QuickQuant™ lateral flow test strips from the desiccant tube and close securely.
6. Place a QuickQuant™ test strip into each test tube with the test strip arrow pointing down.
7. Incubate at room temperature for 10 minutes.
8. Read results **after 10 minutes and before 20 minutes**. For interpretation, refer to Figure 2.

Please note: The line produced by the Mouse IgG Subclass Standard (IgG_1) may be in a different location on the lateral flow strip than the test line produced by the samples. This has no effect on the test results.

Limitations of the Test

Results determined by the lateral flow assay are semi-quantitative. They reflect a relative IgG concentration to the standard. The accuracy of the test may be improved by substituting a purified, concentration-verified and production-specific antibody for the supplied Mouse IgG Subclass Standard.

Variation in purification procedures may result in significant differences in antibody yield from that determined using the QuickQuant™ kit.

For results producing a lower degree of color intensity, the Secondary Sample Dilution (refer to Table A) may be reduced to yield a sample with a higher concentration for testing.

Interpretation of the Results

Visual reading of lateral flow tests

Read strip results after 10 minutes of reaction time but not after 20 minutes. See Figure 1 for an example of representative results. Actual results will vary based on specific cell culture conditions.

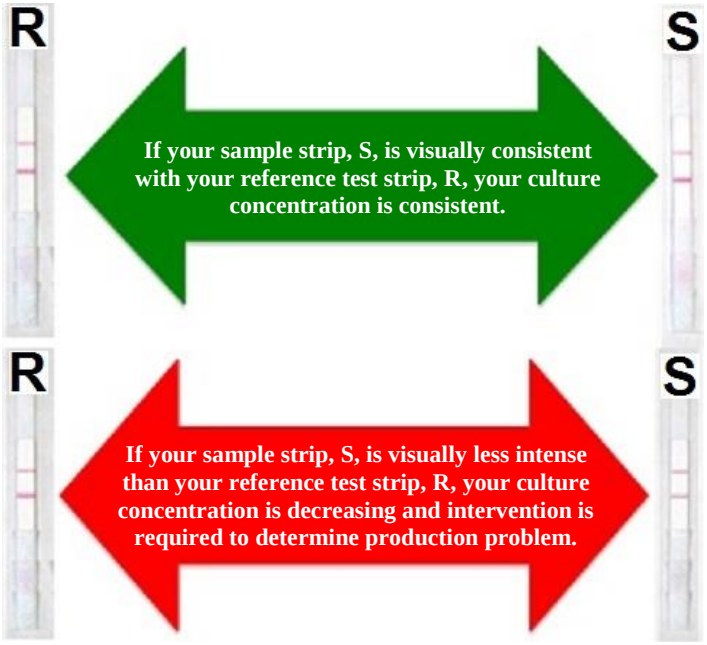

Figure 2: Comparative test procedure.

Archive Sample Preparation

Ongoing testing is improved by running an archived sample along with each new batch of samples. Retention of a 50 µL aliquot (minimum) of archive sample to be used in future comparative testing is recommended. Supernatant fluid samples should be centrifuged to remove cells and cellular debris. A preservative may be added, such as 0.02% sodium azide, to prevent microbial growth. Samples with preservative may be stored at 2-8°C for several months. For long-term or unpreserved storage, keep frozen, ≤ -20°C.

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