KPL TECHNICAL SERVICE REPORT

Stability of Lyophilized Unlabeled Goat Antibodies

Purpose

To measure the performance of lyophilized unlabeled goat antibodies when stored at 2–8°C over a period of 8–10 years.

Materials and Methods

Representative samples of two different goat antibodies were tested. The following lots of BacTrace® Goat Anti-*E. coli* O157:H7 Antibody and Goat Anti-Human IgM (µ) Antibody were stored lyophilized at 4°C (recommended storage) from the date of manufacture. These studies were conducted in August 2010 and October 2010, respectively.

BacTrace Goat Anti-*E. coli* O157:H7 Antibody

Lot No.	Date of Manufacture
WH083	09-01-99
YE104	07-10-01
ZL049	05-02-03
030924	06-12-02
040934	02-08-05
050564	07-08-05
060816	02-09-07
071282	12-14-07
080227	01-18-08
090178	02-09-09
100219	03-08-10
100663	07-20-10

Goat Anti-Human IgM (µ) Antibody

Lot Number	Date of Manufacture
ZL036	10-28-02
030255	02-13-03
040492	06-04-04
050450	03-13-05
060733	06-16-06
070242	03-16-07
080419	04-07-08
081196	10-20-08
100656	07-02-10

The samples were evaluated using a microwell ELISA procedure. BacTrace Goat Anti-*E. coli* O157:H7 unlabeled antibodies were used to detect varying amounts of *E. coli* positive control, while unlabeled Goat Anti-Human IgM (µ) unlabeled antibodies were used to detect varying amounts of purified human IgM. After colorimetric detection, O.D. and slope comparisons were made using a linear regression program comparing a reference lot to the test lots.

The assays were performed as follows at room temperature.

BacTrace Goat Anti-*E. coli* O157:H7 Antibody

1. Nunc MaxiSorp® high binding plates were coated with KPL's BacTrace *E. coli* O157:H7 Positive Control (KPL Catalog No. 50-95-90) diluted 1:100 in 0.01 M PBS at pH 7.4 with 100 µL/well. Following a 1-hour incubation, the plates were emptied and blocked for a minimum of 3 min with 1% BSA Diluent/Blocking Solution (KPL Catalog No. 50-61-00), using a volume of 200 µL/well. The block was emptied and replaced with a 2% sucrose solution (200 µL/well) for a 5-min incubation. The plate was emptied and dried for a minimum of 2 hours inverted.

2. BacTrace Goat Anti-*E. coli* O157:H7 Antibody test samples (KPL Catalog No. 01-95-90) were serial diluted in 1% BSA. Dilutions were started at 2 µg/mL in Row A and ended at 0.0156 µg/mL in Row H. A reference lot was used for comparison. The samples were added at 100 µL/well and allowed to react for 30 min.

3. Plates were washed 3 times with a 3-min soak in between each wash using 1X Wash Solution (KPL Catalog No. 50-63-00).

4. A biotinylated monoclonal antibody against goat IgG was added at 100 µL/well in 1% BSA Diluent/Blocking Solution for 30 min.

5. Plates were washed as in step 3.

6. Peroxidase-labeled Streptavidin (KPL Catalog No. 14-30-00) was diluted to 125 µg/mL in 1% BSA and added at 100 µL/well and allowed to incubate for 30 min.

7. Plates were washed as in step 3.

8. ABTS® Substrate (KPL Catalog No. 50-66-01) was added at 100 µL/well and allowed to react for 15 min.

9. Plates were stopped with 100 µL of 1X ABTS Peroxidase Stop Solution (KPL Catalog No. 50-85-02) per well.

10. The plates were read at 405 nm using an ELISA reader.

11. The potency was determined for each lot by comparing the slope of the reference sample to that of the test samples.

Goat Anti-Human IgM (µ)

1. Nunc MaxiSorp® high binding plates were coated with human IgM antigen diluted in 0.01 M PBS, pH 7.4 at a concentration of 10 µg/mL. Each well received 100 µL of solution. Following a 1-hour incubation the plates were emptied. They were blocked using 1% BSA Diluent/ Blocking Solution (KPL Catalog No. 50-61-00) at a volume of 200 µL/well for 3 min. The plates were emptied, and a 2% sucrose solution was added at a volume of 200 µL/well and allowed to incubate for 5 min. The plates were emptied and allowed to dry at room temperature for 2 hours inverted.

2. Goat Anti-Human IgM (µ) (KPL Catalog No. 01-10-03) test samples were serially diluted in 1% BSA. Dilutions were started at 200 ng/mL in Row A and ended at 1.5625 ng/mL in Row H. A reference lot of Goat Anti-Human IgM (µ) was used for comparison. The samples were added at 100 µL/well and allowed to react for 30 min.

3. Plates were washed 3 times with a 3-min soak in between each wash using 1X Wash Solution (KPL Catalog No. 50-63-00).

4. A biotinylated monoclonal antibody against goat IgG was added at 100 µL/well in 1% BSA Diluent/Blocking Solution for 30 min.

5. Plates were washed as in step 3.

6. Peroxidase-labeled Streptavidin (KPL Catalog No. 14-30-00) was diluted to 125 µg/mL in 1% BSA and added at 100 µL/well and allowed to incubate for 30 min.

7. Plates were washed as in step 3.

8. ABTS Substrate (KPL Catalog No. 50-66-01) was added at 100 µL/well and incubated for 15 min.

9. Plates were stopped with 100 µL/well of 1X ABTS Stop Solution (KPL Catalog No. 50-85-02).

10. Plates were then read at 405 nm using an ELISA reader.

11. The potency was determined for each lot by comparing the slope of the reference sample to that of the test samples.

Results

The data were rationalized using KPL's analysis. In order to pass, the test lot sample must be ≥0.5 of the reference lot. Data are shown below.

*Figure 1. Pass/Fail data for different lots of unlabeled BacTrace Goat Anti-*E. coli *O157:H7 Antibody.*

Lot No.	Date of Manufacture	Pass/Fail
WH083	09-01-99	Pass
YE104	07-10-01	Pass
ZL049	05-02-03	Pass
030924	06-12-02	Fail
040934	02-08-05	Pass
050564	07-08-05	Pass
060816	02-09-07	Pass
071282	12-14-07	Pass
080227	01-18-08	Pass
090178	02-09-09	Pass
100219	03-08-10	Pass
100663	07-20-10	Pass

Figure 2. Pass/Fail data for different lots of unlabeled Goat Anti-Human IgM (µ) Antibody.

Lot No.	Date of Manufacture	Pass/Fail
ZL036	10-28-02	Fail
030255	02-13-03	Pass
040492	06-04-04	Pass
050450	03-13-05	Pass
060733	06-16-06	Pass
070242	03-16-07	Pass
080419	04-07-08	Pass
081196	10-20-08	Pass
100656	07-02-10	Pass

Conclusion

This study shows that the majority of the goat polyclonal antibodies tested continue to perform at a level that would pass quality control specifications 8–10 years after date of manufacture. It may then be concluded that KPL goat antibodies are stable for at least 8 years when stored at 2–8° C in a lyophilized form.

This study also suggests that some lots of antibody remain stable through 10 years from date of manufacture, while others begin to lose performance after 8 years.

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