SOP: Quantitation of CHO DP-12 derived Mouse anti-Human IL-8 Monoclonal Antibody by ELISA

Approvals

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1. Purpose

1.1. Quantitative determination of the concentration and/or titer of CHO-DP12 derived Mouse anti-Human IL-8 monoclonal antibodies.

2. Scope and Applicability

2.1. This ELISA Assay may be used for quantitative determination of Mouse anti-Human IL-8 monoclonal antibodies in cell culture media and chromatography buffers. This assay can be run using a standard curve to calculate antibody concentration or without a standard curve to determine antibody titer.

3. Summary of Method

3.1. Coat 96 well plate with human IL-8
3.2. Block coated 96 well plate
3.3. Preparation of standard
3.4. Preparation of unknown dilutions
3.5. Standard and unknown addition and incubation
3.6. Secondary antibody addition and incubation
3.7. Substrate incubation
3.8. Addition of Stop solution
3.9. Measurement
3.10. Calculation of results

4. References

4.1. Antibodies a Laboratory Manual; Ed Harlow, David Lane
4.2. ELISA Handbook https://www.bosterbio.com/ebooks
4.3. Bio Rad iMark Microplate Absorbance Reader SOP

5. Precautions

5.1. None

6. Responsibilities

6.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
6.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

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7. Equipment and Materials

7.1. Fisher brand polystyrene 96well plate, catalog #12565501 or equivalent.
7.2. Bovine Serum Albumin, (BSA), Fisher catalog #BP9703-100, or equivalent.
7.3. 1X PBS, Hyclone catalog # SH30256.01
7.4. Human IL-8, Rockland, IMAC purified, p/n Custom, or equivalent.
7.5. Rabbit anti-Mouse IgG peroxidase conjugated antibodies, Rockland p/n 6104302, or equivalent.
7.6. TMB, Invitrogen REF # SB01, or equivalent.
7.7. 20µl, 200µl, and 1000µl pipettes and tips
7.8. Shaking platform capable of reaching 300rpm
7.9. Bio Rad iMark Microplate Absorbance Reader
7.10. Microtubes and rack
7.11. Blocking Buffer (3% BSA (w/v) in 1X PBS buffer (pH 7.4))
7.12. 1N HCl
7.13. Anti-Human IL-8 antibody Samples from Spinner Flask and Bioreactor

8. Procedure

8.1. Coat the number of required wells of a polystyrene 96 well plate with Human IL-8 at 800ng/well.
  8.1.1. Prepare enough of an 8ng/µl IL-8 in 1X PBS coating solution for the required number of wells, use 100µl for each well. To coat 20 wells in a 86 well plate prepare 2,000µl, (16µl of a 1mg/ml IL-8 stock and 1,984ul of 1X PBS).
  8.1.2. Pipette 100µl of the coating solution into each well of the 96 well plate.
  8.1.3. Seal the plate and incubate overnight at 4°C on a shaking platform capable of reaching 300rpm.
  8.1.4. inverte plate over the sink to remove the coating solution and then tapped on paper towels
  8.1.5. wash plate twice using 250µl of 1X PBS per well, invert the plate and tap as above to remove the wash buffer from the wells
8.2. Block the coated wells of the 96 well plate
  8.2.1. Pipette 250µl of Blocking Buffer, (3% w/v BSA in 1X PBS), into each of the coated wells of the plate
  8.2.2. Seal the plate and incubate for 2 hours at room temperature on a shaking platform capable of reaching 300rpm.(blocking step may also be incubated overnight)
  8.2.3. Wash plate as above three times using 250µl of 1X PBS per well.
8.3. Preparation of Standard and Sample solutions; (Dilutions for the standard curve and zero standard must be made and applied to the plate immediately)
  8.3.1. From a 10ug/ml anti-IL-8 mAb standard solution prepare 300ul of a 800ng/ml, 600ng/ml, 400ng/ml and 200ng/ml solution in Blocking Buffer
  8.3.2. Follow dilution table located in attachments section for standard preparation.
8.4. Make 300µl of an appropriate dilution in blocking buffer for each sample (typically 1:10, 1:100 or 1:1000 dilutions are used)

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8.5. Uncover the coated and blocked microtiter plate and remove the blocking solution by inverting the plate over the sink. Wash the plate 3times with 1X PBS as above.

8.6. Add 100µl of anti-IL-8 mAb standards in duplicate wells and media samples in single wells and cover the plate. Be sure to carefully record their position on the microtiter plate and record in SOP plate template.

8.6.1. Shake plate at 300rpm for 1 hour.

8.6.2. Wash wells three times with 250µl of 1X PBS. Remove excess wash buffer by gently tapping microtiter plate on a paper towel or Kimwipe.

8.7. Secondary Antibody Addition (Rabbit anti-Mouse IgG).

8.7.1. Prepare a 1:1,000 dilution of the Rabbit anti-Mouse IgG antibody.

8.7.2. Add 100µl of secondary antibody to all wells and cover the plate.

8.7.3. Shake plate at 300rpm for 1 hour.

8.7.4. Wash wells three times with 250µl of 1X PBS. Remove 1X PBS by gently tapping microtiter plate on a paper towel or kimwipe.

8.8. Substrate Incubation

8.8.1. Add 100µl of TMB substrate to all wells and shake at 300rpm for approximately 2-10 minutes. Substrate will change from colorless to different shades of blue.

8.8.2. Quench reaction by adding 100µl of 1N HCl in the same order as the substrate was added to stop the reaction. The color will change from blue to yellow. Mix thoroughly by gently shaking the microtiter plate by hand for approximately one minute.

8.9. Measurement

8.9.1. Measure the absorbance in all wells at 450nm using the Bio Rad iMark Microplate Absorbance Reader.

8.10. Calculation of Results

8.10.1. Subtract the value of the zero point standard from all of the standards and unknowns to determine the corrected absorbance (A_{450}).

8.10.2. Plot A_{450} against the amount of anti-IL-8 mAb in the standards (possibly on a log scale), to create a anti- IL-8mAb standard curve.

8.10.3. Use a 3rd order polynomial best fit or 4-parameter fit to plot the points of the anti-IL-8 mAb standard curve.

8.10.4. The amount of anti-IL-8 mAb in the unknowns can be determined from the slope of the standard curve.

8.10.5. Create a graph showing concentration of anti-IL-8 mAb over time in days.

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9. Attachments

Anti-IL-8 mAb Concentration (ng/ml)	Dilutions
0	250µl (blocking buffer) Zero point to determine background
200	
400	
600	
800	
1000	

Figure 1. Dilution table for preparation of anti-IL-8mAb standard

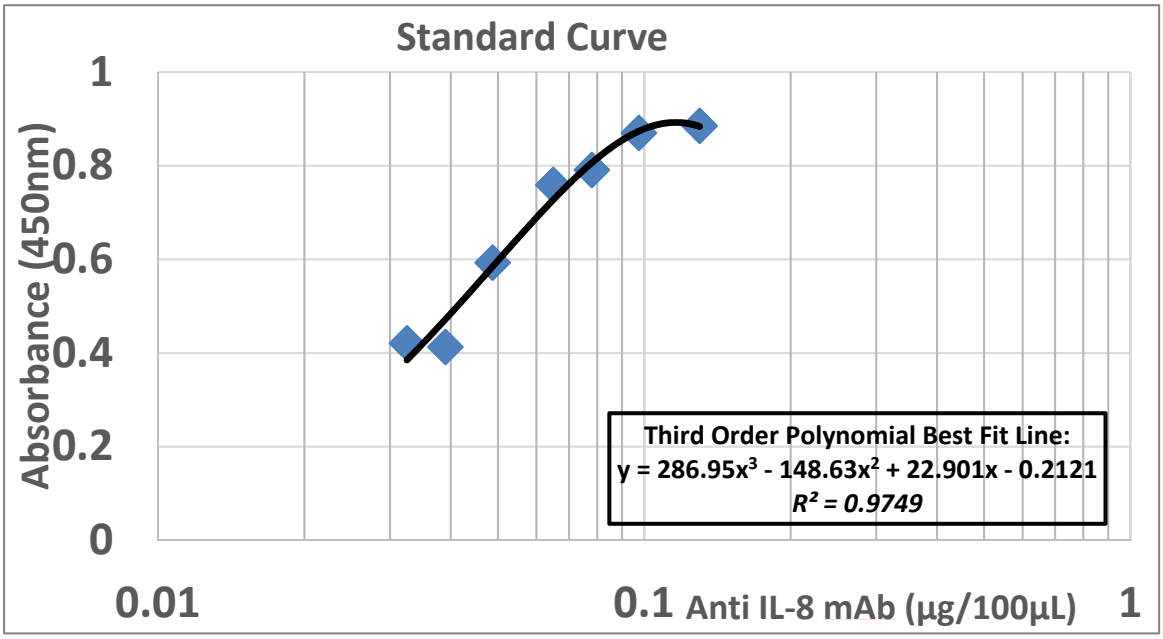

Figure 2. Anti-IL-8 mAb standard curve (Example Only)

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	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Figure 5. ELISA Plate Layout

10. History

Revision Number	***Effective Date***	***Preparer***	***Description of Change***
0	07JUL2017	Robin Zuck	Initial release
1	05SEP2017	Hetal Doshi	Optimization of methods