SOP: Quantitation of CHO DP-12 Derived Anti 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 Humanized Mouse anti-Human IL-8 monoclonal antibodies.

2. Scope and Applicability

2.1. This ELISA Assay may be used for quantitative determination of Humanized 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 test sample dilutions
3.5. Standard and test sample 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, Publisher: Cold Spring Harbor Laboratory Press
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 96 well 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 or equivalent.
7.4. Human IL-8, without BSA or other protein stabilizer.
7.5. Rabbit anti-Human IgG peroxidase conjugated antibody, ThermoFisher catalog # PA1-28587, or equivalent.
7.6. TMB, Invitrogen REF # SB01, or equivalent.
7.7. 20µl, 200µl, 1000µl pipettes and tips
7.8. A 30-300µl multichannel pipette and tips, if available.
7.9. Shaking platform capable of reaching 300rpm.
7.10. Bio Rad iMark Microplate Absorbance Reader.
7.11. Microtubes and rack
7.12. Blocking Buffer (3% BSA (w/v) in 1X PBS buffer (pH 7.4))
7.13. 1N HCl
7.14. Purified anti IL-8 mAb to use as a standard
7.15. Anti-Human IL-8 antibody Test Samples from Spinner Flask, Bioreactor and Chromatography steps

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 96 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. Invert the plate over the sink to remove the coating solution and then tap on paper towels to be sure all the coating solution is removed.
8.1.5. Wash the plate twice using 250µl of 1X PBS per well, invert the plate and tap on paper towels 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 or overnight at 4°C, on a shaking platform capable of reaching 300rpm.
8.3. Preparation of Standard and Test Sample solutions; (Dilutions for the standard curve and samples should be prepared before removing the blocking solution.)

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8.3.1. From a 1ug/ml anti IL-8 mAb standard solution prepare 500ul of a 120ng/ml, 100ng/ml, 80ng/ml, 60ng/ml, 40ng/ml, 20ng/ml and 10ng/ml solutions in Blocking Buffer.

8.3.2. Follow the dilution table located in attachments section for standard preparation.

8.4. Prepare 300µl of an appropriate dilution in blocking buffer of each test sample (typically 1:10, 1:100 or 1:1000 dilutions are used).

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

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

8.6.1. Shake plate at 300rpm for 1 hour at room temperature.

8.6.2. Wash the plate three times as above with 1X PBS.

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

8.7.1. Prepare a 1:16,000 dilution of the Rabbit anti-Human IgG antibody in blocking buffer.

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 at room temperature.

8.7.4. Wash wells three times as above with 1X PBS.

8.8. Substrate Incubation

8.8.1. Add 100µl of TMB substrate to all wells and shake at 300 rpm for approximately 3-6 minutes, until a color gradient is observed in the standard wells, (usually 3-6 minutes). See Figure 3.

8.8.2. Stop the reaction by adding 100µl of 1N HCl in the same order as the substrate was added to the wells. The color will change from blue to yellow. Mix thoroughly by gently tapping the microtiter plate. The color in each well should appear uniform.

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_{450nm}).

8.10.2. Calculate the average of the duplicate standard wells.

8.10.3. Plot A_{450nm} against the concentration of anti-IL-8 mAb in the standards, to create an anti IL-8 mAb standard curve.

8.10.4. Use a linear fit to plot the points of the anti-IL-8 mAb standard curve.

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

8.10.6. For upstream processing, create a graph showing the concentration of anti-IL-8 mAb of the Culture in days.

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8.10.7. For downstream processing, determine the concentration of anti IL-8 mAb in pre and post Protein A chromatography samples.

Note: The 120ng/ml point may not fall within the linear portion of the curve. If this point is not in the linear portion of the curve remove it from your standard curve.

9. Attachments

Anti IL-8 mAb Concentration (ng/ml)	Dilutions
0	100µl/well (blocking buffer) Zero point to determine background
10	
20	
40	
60	
80	
100	
120	

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

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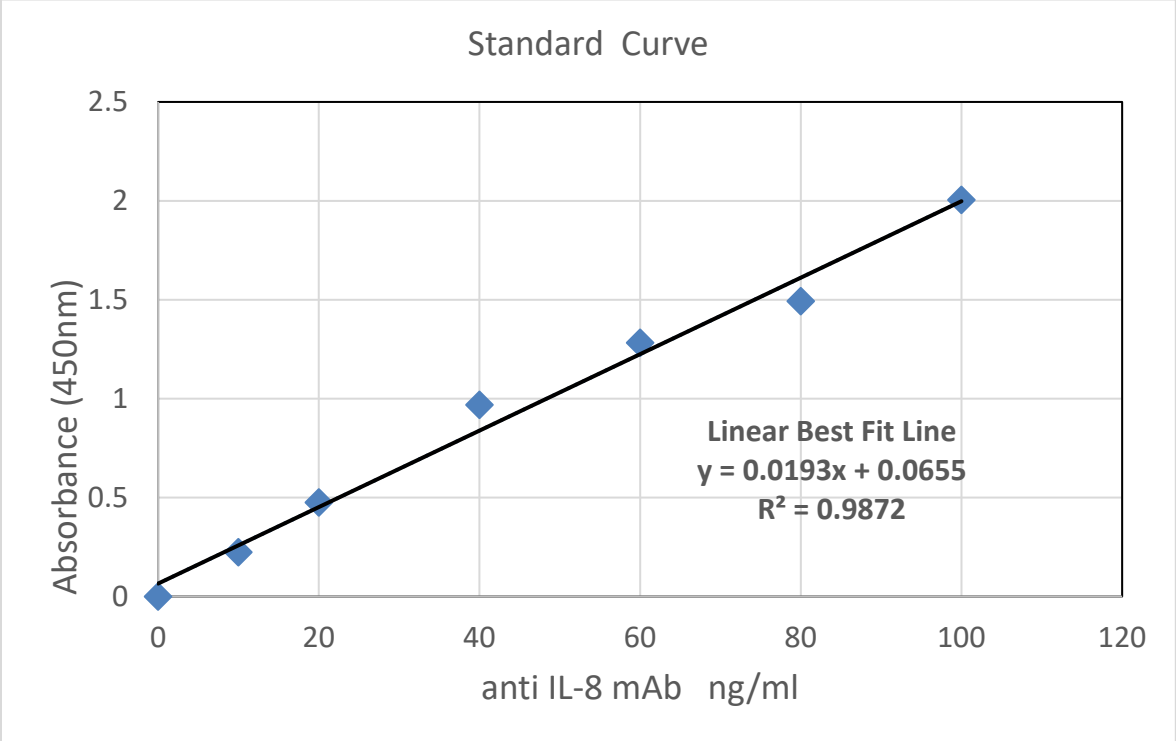

Figure 2. Anti IL-8 mAb standard curve (Example Only)

0	10	20	40	60	80	100	120ng/ml

Figure 3. Anti IL-8 mAb standard curve wells before addition of Stop solution.

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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	13JAN2018	Robin Zuck	Change secondary antibody to Rb anti Human IgG Edited title to include “Humanized Mouse Anti IL-8 Antibody”