Product datasheet

Note: This protocol was modified as of July 2017. All previous protocols are obsolete.

Human GM-CSF ELISA Kit

Product #: 0008

Storage recommendations

Store the kit at 2-8°C. The kit is stable for a period of up to 3 months from the date of receipt.

Description

This human GM-CSF ELISA kit is designed to enable the quantitative measurement of natural and/or recombinant GM-CSF in serum, plasma and cell culture media. The kit contains one strip-well plate pre-coated with human GM-CSF capture antibody along with sufficient key reagents for plate development.

Reagent preparation

Wash buffer (20x)

If crystals have formed in the concentrate, warm to room temperature and mix well. Once all the visible crystals have dissolved, dilute 25mL of wash buffer concentrate in 475mL of distilled water to make 500mL of wash buffer.

Standard

The standard is present in lyophilised form with the amount of standard present in the tube clearly stated on the label. Prior to reconstitution, briefly centrifuge the vial to ensure that any loose lyophilised material has collected at the bottom of the tube.

For cell culture samples:

Reconstitute the standard in 1mL of assay diluent 1B and mix well by inversion. Allow the standard to dissolve for at least 15 minutes with regular inversion prior to use. Dividing the amount of lyophilised standard (as per the label) by 1mL will give the standard concentration. Use sample culture medium to perform the serial dilutions.

For serum/plasma samples:

Reconstitute the standard in 1ml of assay diluent 1B and mix well by inversion. Allow the standard to dissolve for at least 15 minutes with regular inversion prior to use. Dividing the amount of lyophilised standard (as per the label) by 1mL will give the standard concentration. Use assay diluent 1B to perform the serial dilutions.

For research purposes only. Not for diagnostic use.
The standard curve should be performed in duplicate starting at 4000pg/mL and diluted 1 in 2 down to at least 62.5pg/mL. Make 1mL of the 4000pg/mL standard in a micro tube ensuring that the appropriate diluent is used. Serial dilutions are easily achieved by using a micro-titre plate (or similar). Load 300µL of the 4000pg/mL standard into the first 2 wells of the dilution plate and load 125µL of the appropriate diluent into the remaining wells. Using a multichannel pipette, transfer 125µL from the first wells (i.e. 4000pg/mL) into the second wells (now 2000pg/mL), mix well by pipetting up and down before transferring 125µL to the next wells (now 1000pg/mL). Repeat until the serial dilution is complete.

**Detection antibody**

Briefly centrifuge prior to use. The anti-human GM-CSF biotin labelled detection antibody is provided in a liquid form at the concentration specified on the tube. The anti-human GM-CSF biotin labelled detection antibody should be diluted to 150ng/mL in assay diluent 1B immediately prior to use.

**Streptavidin-HRP conjugate**

Briefly centrifuge prior to use. The streptavidin-HRP conjugate should be diluted 500 fold in assay diluent 1B immediately prior to use. For example, if developing the entire plate aliquot 22µL of streptavidin-HRP conjugate into 11mL of assay diluent 1B.

**Protocol**

It is highly recommended that all standards, samples and zero standard controls be performed in duplicate. Ensure that all reagents are at room temp prior to use.

1. Prepare solutions as required by following the instructions outlined in the reagent preparation section.
2. Remove any 8 well strips that are not required for the assay and place back in the foil pouch ensuring that it is closed tightly.
3. Add 50 µL of assay diluent 1B to each well.
4. Add 50µL of standards, samples and zero standard controls to each well and seal the plate with the adhesive cover provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash the plate 4x with wash buffer (250µL). Washing can be achieved with a multichannel pipette, plate washer or similar. To remove residual wash buffer invert the plate and tap the plate on paper towels between each wash.
6. Add 100µL of biotin labelled detection antibody to each well and seal the plate with the adhesive cover provided. Incubate for 2 hours at room temperature.

7. Repeat the aspiration/wash steps in step 5.

8. Add 100µL of freshly diluted streptavidin-HRP conjugate to each well and seal the plate with the adhesive cover provided. Incubate for 45 minutes at room temperature.

9. Aspirate each well and wash the plate 5x with wash buffer (250µL) as per step 5. It is important to wash thoroughly here to reduce unwanted background.

10. Add 100µL of TMB substrate to each well and incubate for approximately 15 minutes at room temperature. **Ensure the plate is protected from light.** Check the development process (blue colour) every 5 minutes or so to prevent over development of the plate.

11. Stop the reaction with 50µL of stop solution. The wells will turn from blue to yellow in colour.

12. Determine the optical density (OD) of the plate using a micro-plate reader set at 450nm. If the micro-plate reader allows wavelength correction, set at 570nm.

**Calculation of results**

Calculate the mean absorbance for each standard, sample and control and subtract the mean of the zero standard controls.

Create a standard curve by plotting the mean OD of each standard on the y-axis versus the standard concentration on the x-axis. If available, use a program capable of generating a four parameter logistic (4-PL) curve fit. A free user friendly 4-PL program is available online at [http://elisaanalysis.com](http://elisaanalysis.com). Alternatively, plot a best fit curve through the standard points and overlay a linear or polynomial trend-line. The resultant equation displayed on the graph can then be used to calculate the unknown concentrations by solving for x. When fitting a trend-line it is essential that the r² value is 0.99 or higher. If not, then simply find the linear portion of the graph by removing the higher standard points from the analysis.

If the samples were diluted then multiply the concentration read from the standard curve by the dilution factor.
Standard curve

This is a representative example of a typical standard curve. A separate standard curve must be generated for each experiment.

![Standard Curve Graph](image)

Sensitivity

The lower limit of quantitation (LLQ) of the assay is typically <5pg/mL.

The LLQ was determined by adding 3 standard deviations to the mean optical density of 5 zero antigen replicates and calculating the corresponding concentration.

Precision.

Intra-assay CV: <10%

Inter-assay CV: <10%

Recovery.

The various matrices were spiked with recombinant human GM-CSF and compared to a spiked assay diluent control. Typical values are as follows.

<table>
<thead>
<tr>
<th>Sample</th>
<th>250pg/ml</th>
<th>125pg/ml</th>
<th>62.5pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell culture</td>
<td>98</td>
<td>77</td>
<td>106</td>
</tr>
<tr>
<td>media</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>86</td>
<td>89</td>
<td>139</td>
</tr>
<tr>
<td>Plasma</td>
<td>70</td>
<td>62</td>
<td>107</td>
</tr>
</tbody>
</table>

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**Linearity.** Linearity of dilution was determined by serially diluting spiked samples and analysing them in the ELISA.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution factor</th>
<th>Expected (pg/ml)</th>
<th>Observed (pg/ml)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell culture media</td>
<td>neat</td>
<td>202</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>101</td>
<td>84</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Serum</td>
<td>neat</td>
<td>178</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>89</td>
<td>97</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>45</td>
<td>48</td>
<td>106</td>
</tr>
<tr>
<td>Plasma</td>
<td>neat</td>
<td>145</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>73</td>
<td>67</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>36</td>
<td>33</td>
<td>92</td>
</tr>
</tbody>
</table>

**Cross reactivity**

The following antigens showed less than 5% cross-reactivity when assayed at 50ng/ml: Human G-CSF, IL-3 sRα, IL-5 sRα and M-CSF. mouse and rat GM-CSF.

**Technical hints**

Always ensure complete reconstitution and/or dilution of reagents prior to use.

To ensure uniform temperature/humidity levels for all steps of the ELISA across the entire plate it is often beneficial to perform the incubations in a humidity chamber. This can simply be a Styrofoam box containing moistened paper hand towels.

Plates should always be incubated with the adhesive plate sealer in place.

Always ensure accurate pipetting of reagents at all times, especially the standards.