PMN- 02 - MTT Assay in Human PMN V.1

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BEFORE STARTING

MATERIALS

<table>
<thead>
<tr>
<th>Material</th>
<th>Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethyl sulfoxide (DMSO)</td>
<td>Sigma</td>
</tr>
<tr>
<td>Fetal Bovine Serum</td>
<td>EuroClone</td>
</tr>
<tr>
<td>RPMI</td>
<td>EuroClone</td>
</tr>
<tr>
<td>Thiazoyl blue tetrazolium bromide (MTT)</td>
<td>Sigma</td>
</tr>
<tr>
<td>Penicillin/Streptomycin</td>
<td>EuroClone</td>
</tr>
</tbody>
</table>

Instrumentation needed:

- Sterile and non-sterile plastic disposables
- ELISA plate reader

Use all reagents at Room Temperature

1. Isolate PMN according to the protocol PMN-01a for buffy coats or PMN-01b for fresh whole blood.

2. Resuspend PMN at 1x10⁶ cells/ml in RPMI 1640 medium supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin/streptomycin.

3. Add 250 µl of cell suspension per well in a 96-well round bottom plate and incubate for 24:00:00 alone or in the presence of the test substance at 37 °C in a 5% CO₂ atmosphere. **Cells must be plated in duplicate for each experimental condition.**

4. Centrifuge at 1400 x g, 00:05:00 and discard the supernatant.

5. Resuspend cells with 200 µl of MTT solution at 0.5 mg/ml, incubate for 02:00:00 at 37 °C in a 5% CO₂ atmosphere.

6. Centrifuge at 1400 x g, 00:05:00 and discard the supernatant.

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7. Add 120 µl of DMSO per well, mix the plate until the formazan crystals are dissolved.

8. Read the plate on the ELISA plate reader at 570 nm.