LPS stimulation of HeLa cells

Material

- HeLa cells transfected the day before stimulation
- Growth medium (see protocol “Growth Medium Preparation”)
- Lipopolysaccharide (LPS, obtained from Sigma Aldrich as powder), 1 mM stock solution

Procedure

All steps are performed in a sterile environment – we always worked within a biosafety cabinet, but using a laminar floow hood or clean bench is also possible.

Transfection of HeLa cells the day before stimulation

1. Transfect HeLa cells with either Gaussia luciferase containing plasmid as described in the protocol “Lipofection”.
   Note: We used plasmids carrying Gaussia luciferase as we used the luciferase as subsequent reporter to analyze the effect of LPS stimulation by performing luminescence assay (see respective protocol).
2. For each plasmid, transfect cells in 4 wells.
   Note: Remember to also keep some untransfected HeLa cells as negative control for subsequent analysis.

LPS stimulation

1. Prepare growth medium containing different concentrations of LPS. Use the following conditions:
   - 0 µg LPS/mL (untreated control)
   - 0.5 µg LPS/mL
   - 1 µg LPS/mL
   - 2 µg LPS/mL
2. Add 250 µL of LPS containing growth medium per well.
3. Incubate cells with LPS for 3 hours at 37°C, 5 % CO_2 and a humidified atmosphere.
4. Remove LPS containing medium.
5. Add fresh growth medium.
6. Incubate cells 37°C, 5 % CO_2 and a humidified atmosphere.
7. Analyze 24 h and 48 h after stimulation.
   Note: For analysis, we performed a luminescence assay.

Notes

- Always wear a labcoat and gloves.
- Clean the hood, your gloves and all the material which you are about to use with 70 % ethanol before starting your work/putting consumables under the sterile hood to prevent any possible contamination.
- Only open the consumables within a sterile environment to keep them sterile and to prevent any possible contaminations.