

24-well plates for assays of cytokine production by ELISA, and 6-well plates for analysis by immunoblotting or reverse transcriptase PCR (RT-PCR).

4. The next day, stimulate the cells with 1–100 ng/ml LPS (usually, 10 ng/ml is enough to observe most responses).
5. After stimulation, collect supernatants for ELISA, prepare whole cell lysates for immunoblotting, or prepare total RNA for RT-PCR.

This experiment is suitable for cell surface TLRs, but not intracellular TLRs. We could not detect any responses to LPS in macrophages expressing intracellular TLR chimeras. LPS does not seem to reach intracellular TLRs.

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