MAT Assay - Using Assay Ready Macrophages Derived from THP-1 Cells

The pyrogen detection assay is an in vitro test method to detect or quantify substances that activate human monocytes to release endogenous mediators such as pro-inflammatory cytokines, like tumor necrosis factor alpha (TNF-α), which play an important role in fever pathogenesis (Fig.1). The assay detects the presence of endotoxins as well as non-endotoxin pyrogens and is capable to replace the rabbit pyrogen test.

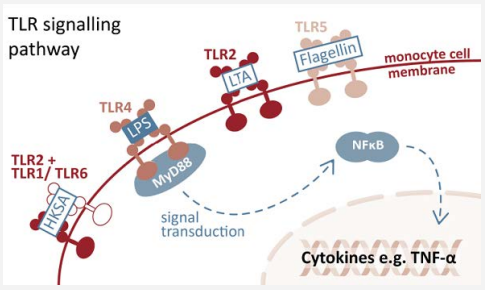

Fig.1: TLR signalling pathway; Toll-like receptors, expressed by macrophages and initiated by pyrogens, start a signal transduction that leads to the expression of cytokines such as TNF-α.

THP-1 derived macrophages were cryopreserved in assay ready format. These prequalified cells can be used like a reagent in the monocyte activation test, i.e. without prior cultivation. Assay Ready Cells are more precise than cells from continuous culture due to lack of variances from cell handling and passaging.

THP-1 macrophages express the differentiation markers CD11b and CD14

THP-1 cells were differentiated into macrophages in the presence of PMA. Macrophages express differentiation markers. CD11b also known as Integrin α-M its function is to promote cell adhesion and phagocytosis. Another marker is the CD14 which binds to lipopolysaccharide.

A flow cytometry analysis confirms the expression of both markers from the macrophages (Fig.3), therefore verifying that the differentiation of the THP-1 cells into macrophages has been successful.

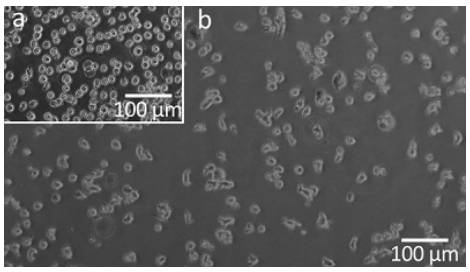

Fig.2: Microscopic image of (a) undifferentiated THP-1 cells and (b) THP-1 cells differentiated into macrophages, the state in which the cells are frozen for Assay Ready Cells.

Method MAT-Assay

The macrophages were seeded into a 96 well plate at a density of 100,000 cells/well and left to adhere for 24 hours. On day 2, a serial dilution of the endotoxin reference standard was added to

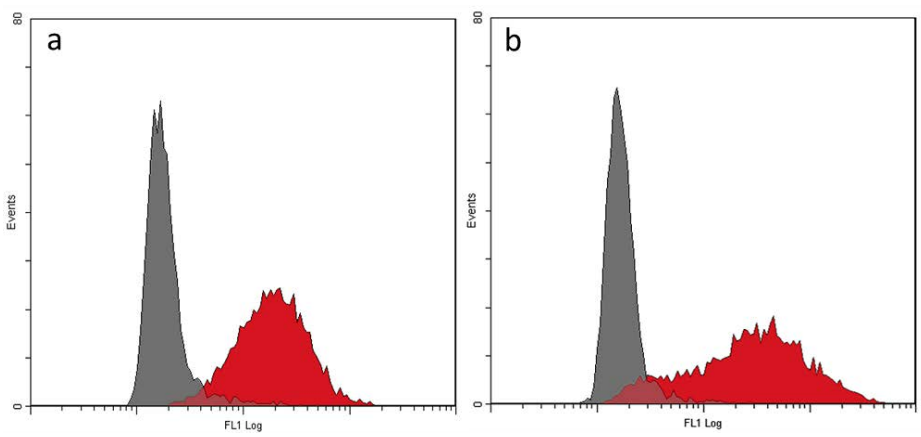

Fig.3: Expression of (a) CD11b and (b) CD14. The values for THP-1 cell are shown in grey, for differentiated macrophages in red.

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the cells and incubated for 18 hours. After the 18-hour incubation period an ELISA was carried out to measure the level of TNF-α secretion.
According to the Ph. Eur. 2.6.30 spike recovery experiments were carried out. Namely, a 1:2 dilutional series of the reference standard endotoxin has been spiked with standard endotoxin at a concentration equal to the middle dose from the endotoxin standard curve.

Assay ready THP-1 macrophages are sensitive and precise

Six different macrophage batches have been compared (Fig. 4) and the results show an inter-assay CV of 16.21% at the LOD level (0.05 EU/ml) and 2.85% at the upper level (0.8EU/ml) of the endotoxin standard curve.

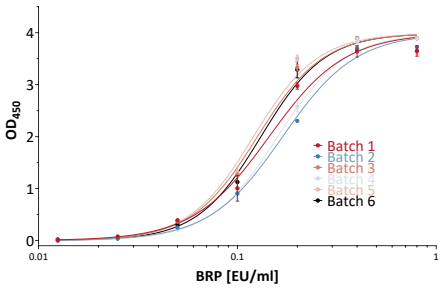

Fig. 4: Batch to Batch Comparison

The spike recovery tests showed an average recovery rate between 71.0% and 107.5%. These results prove that the batches are precise and accurate on a reproducible level.

Non endotoxin pyrogens can also be detected

In addition to the endotoxin LPS, the macrophages also react to non endotoxin pyrogens, such as HKSA, Flagellin or LTA as shown in figure 5. Concluding, the macrophages express the toll-like receptors TLR5 (detection of Flagellin), TLR2 (detection of LTA) and TLR1/2/6 (detection of HKSA).

Assay ready THP-1 macrophages are as sensitive as cultured cells

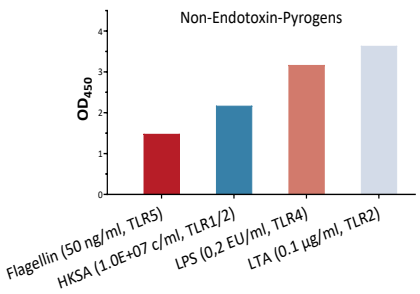

Fig. 5: Detection of non-endotoxin pyrogens.

Differentiated cells from a continuous culture and assay ready cells which were directly seeded into the assay plate, as seen in figure 6, perform similarly. This can also be seen in the hill slope values with continuous cells at 2.99 and assay ready cells at 2.50.

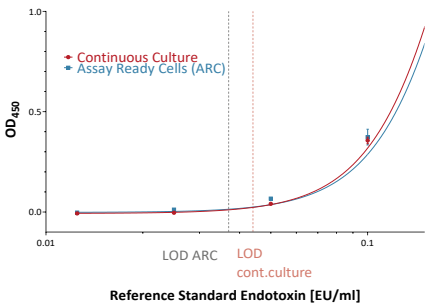

Fig. 6: Sensitivity of assay ready THP-1 macrophages.

conclusion

The assay ready macrophages fulfil all criteria to run a reliable, precise, and accurate monocyte activation assay. Not only are macrophages a great replacement of the rabbit pyrogen test because of their biological abilities but because of the easy and safe handling of them for measuring pyrogen contaminations.

related products

instaCELL pyrogen detection kit

validated kit with prequalified Assay Ready Macrophages, media & buffer, Endotoxin Standard, TNF-α ELISA, 96-well plates (CatN° SF240-01)

Assay Ready THP-1 macrophages

prequalified cells for instant use, no propagation required.

Including recovery buffer and assay medium; 10 million cells/vial (CatN°R514)