

## CELL-BASED ASSAYS

### Proinflammatory cytokine production/release

Cytokines are key modulators of inflammation, participating in acute and chronic inflammation via a complex and sometimes seemingly contradictory network of interactions. Better understanding of how these pathways are regulated helps facilitate more accurate identification of agents mediating inflammation and the treatment of inflammatory diseases. Key pro-inflammatory cytokines include interleukin-1 (IL-1), IL-6 and tumour necrosis factor (TNF- $\alpha$ )<sup>1</sup>.

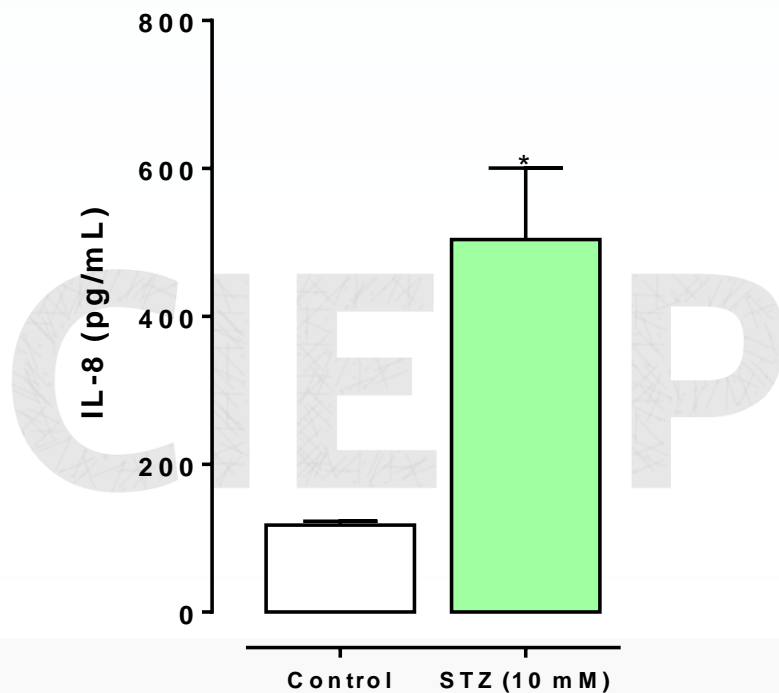
**Test system:** Primary Cells or Immortalized Cell Lines

**Experimental number:** Three wells per group in triplicate.

**Reference Item:** Upon request.

**Main Read-outs:** IL-1 $\beta$ , TNF- $\alpha$ , IL-8, IL-6, IL-10, TGF- $\beta$ , among others.

### Validation Data



**Figure:** Evaluation of IL-8 levels in cell culture. The figure show IL-8 (pg/mL) quantification after streptozotocin-stimulated glioblastoma cells (STZ, 10 mM) for 24 hours and compared with control group (vehicle). Each column represents the mean  $\pm$  SEM of 3 wells per group in triplicate. Statistical analyses used was *t*-test. \*P < 0.05 versus vehicle group (control).

To avoid bias and to allow reproducibility and reliability of all *in vitro* experiments we follow the “Guidance on Good Cell Culture Practice”<sup>2</sup>. All *in vitro* experiments were performed in triplicate wells for each condition and repeated at least three times.

#### References:

<sup>1</sup>Turner MD, Nedjai B, Hurst T, Pennington DJ. Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. *Biochim Biophys Acta*. 2014 Nov;1843(11):2563-2582.

<sup>2</sup>Coecke S; Balls M; Bowe G; Davis J; Gstraunthaler G, Hartung T, Hay R, Merten OW, Price A, Schechtman L, Stacey G, Stokes W. Guidance on good cell culture practice: a report of the second ECVAM task force on good cell culture practice. *Altern Lab Anim*. 2005, 33(3):261-87.