In vitro Inflammation Model induced by Cytokines

Reference:

Enríquez-de-Salamanca A., Calder V., Gao J., Galatowicz G., García-Vázquez C., Fernández I., Stern M.E., Diebold Y., Calonge M. Cytokine responses by conjunctival epithelial cells: An in vitro model of ocular inflammation. Cytokine, 2008; 44: 160-167.

Fundamentals:

The ***In vitro*** **Inflammation Model induced by Cytokines** allows identifying the presence of an inflammatory process in cell cultures through an increased expression of proinflammatory metabolites such as IL-6, following exposure to cytokines such as TNF-α.

Technique:

Seed Human Corneal Epithelial (HCE) or Normal Human Conjunctiva (IOBA-NHC) cells in a 24-well plate (80,000 cells/well in 500 µl culture medium/well). Gently shake plates after seeding in order to homogenize cell distribution over the well surface. Let them grow for 48 h at standard conditions (37 ºC, 5% CO_2/95% air atmosphere).

Replace HCE or IOBA-NHC culture media for serum-free, nonsupplemented medium (DMEM/F-12). Maintain cells in the incubator for 24 h.

Afterwards, set experimental conditions:

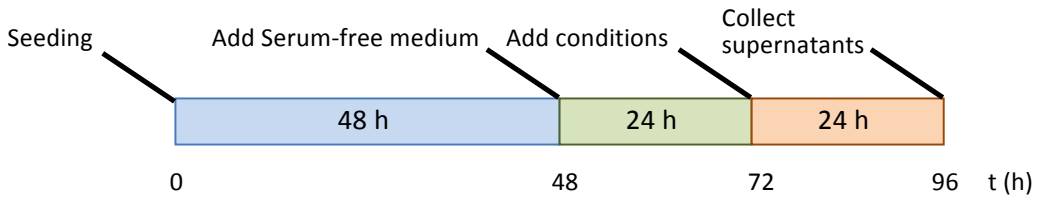
> Positive control: TNF-α in serum-free, nonsupplemented medium (25 ng/ml). Use a handy TNF-α concentration in mother solution like 100 µg/ml to prepare the working solution.
>
> Negative control: serum-free, nonsupplemented medium.
>
> Samples: in serum-free, nonsupplemented medium.

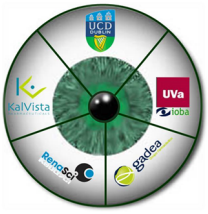
Maintain cells in the incubator for 24 h.

Collect cell culture supernatants and maintain at −80 °C until use.

Quantify IL-6 secretion using the human IL-6 ELISA Kit.

Timeline:





Note: HCE and IOBA-NHC media are described in "HowToDo-human ocular cell lines".