

Cell-Based Assays for Interferons

Cell-based activity assays are indispensable tools in lead identification, process development and API release-testing. ProBioGen offers analysis and comparison of Interferons, e.g. for their potency and mode-of-action, with a range of established and highly reliable assays.

Interferons (IFNs) are categorized into three classes, designated type I - III, according to their respective receptor complexes. Type I Interferons comprise the IFNs- α and - β , both binding to the IFN- α receptors. However, they possess different binding affinities, resulting in differently pronounced effects *in vivo* and *in vitro*.

When Interferons were discovered more than 50 years ago, their anti-viral activity was observed first. This is also reflected in the European Pharmacopoeia (EP) which states the Anti-Viral Assay (AVA) in monograph 1110 for potency testing of IFN- α . Today, several additional properties such as immunosuppression and other immunological effects, as well as the regulation of cell proliferation and differentiation are known. This calls for additional cell-based assays for investigating bioactivity and potency, especially of biosimilars, second generation products or modified Interferons.

Our Interferon assay portfolio covers:

- ProBioGen's accurate reporter gene assay for type I Interferons, using the MxA promoter (recommended by Meager, 2002). This dual luminescence assay combines the ISRE-induced luminescence with a housekeeping gene control
- Anti-Viral Assays (AVA), according to the European Pharmacopoeia, using the A549 cell line in combination with EMCV
- MHC-I up-regulation assays for immuno-modulating substances
- Commercially available reporter gene assay iLite alpha/beta (Biomonitor), using the ISG15 promotor
- Anti-proliferation assays with various cell lines (e.g. WISH, Daudi)
- Anti-cytokine assay, measuring the GM-CSF interfering effect of type I Interferons on the TF-1 cell line (Mire-Sluis et al., 1996).

- ProBioGen's Induced Cytokine Release Assay (ICRA) to investigate the cytokine responses (bead array) of antigen-stimulated human PBMCs.

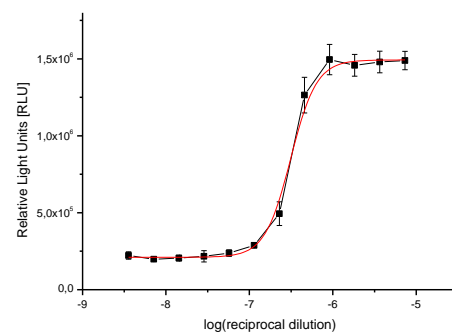
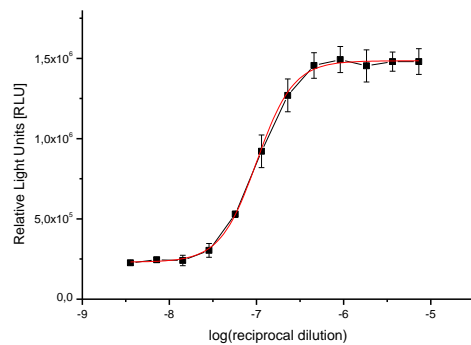


Figure 1: Anti-viral assay (AVA). The assay was conducted according to the EP, using EMC virus in combination with the A549 cell line, measuring cell survival. Compared is a second generation IFN- β (top) with a commercial IFN- β product (bottom). The decreased slope in the inflexion point shows lower IFNAR1 affinity of the second generation IFN- β .

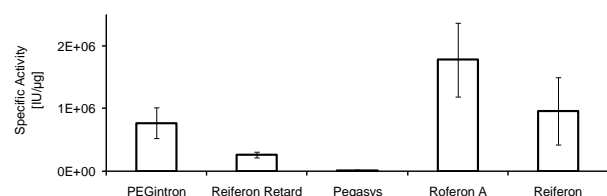


Figure 2: Comparison of different Interferons. PEGylated (PEGIntron, Reiferon Retard, Pegasys) and non-PEGylated IFN- α (Roferon A, Reiferon) were analyzed in a commercial type I Interferon reporter gene assay.

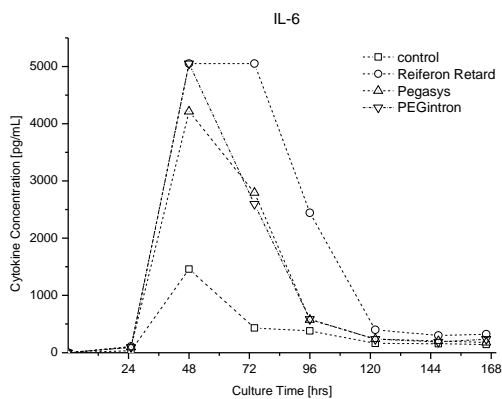
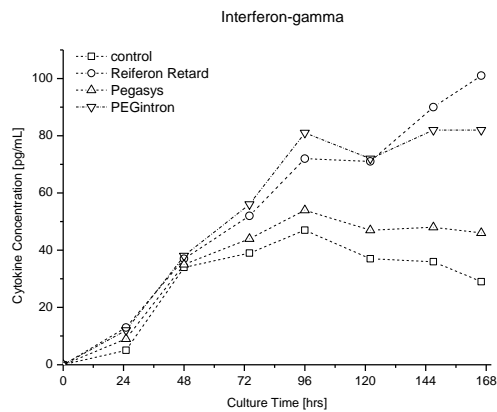


Figure 3: Selected cytokine responses (IFN- γ and IL-6) of viral antigen (HAV) stimulated human PBMCs cultivated with physiological concentrations of PEGylated IFN- α products. The increased secretion of the pro-inflammatory cytokine IFN- γ indicates the induction of a cellular immune response. In contrast, the increased and prolonged IL-6 secretion indicates the potential to induce a humoral immune response.

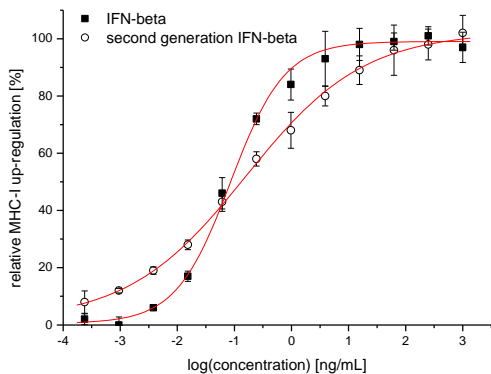


Figure 4: Comparison of the immune-modulating effect (MHC-I up-regulation) of IFN- β . Shown are a second generation IFN- β and a commercial IFN- β product. The MHC-I up-regulation was analyzed by flow cytometry.

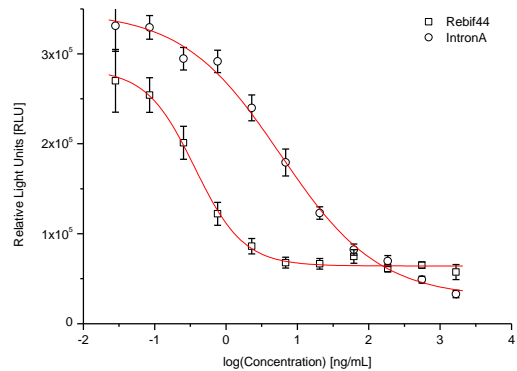


Figure 5: Anti-proliferation assay. An IFN- α and an IFN- β product were compared, using the interferon-sensitive WISH cell line. The 4pl curves show a more potent anti-proliferative effect of IFN- β .

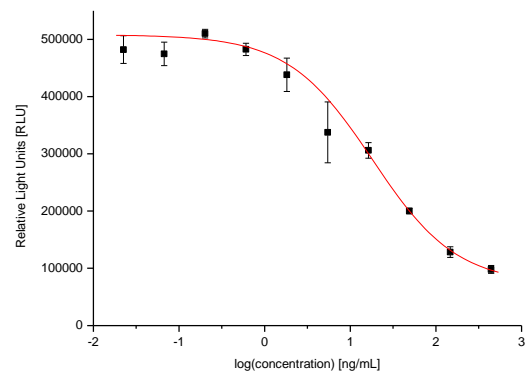


Figure 6: Anti-Cytokine Assay. The GM-CSF interfering effect of IFN- α on the cell line TF-1 was investigated in this assay.