222-M A NEW ELISPOT/FLUOROSPOT BEST PRACTICE USING A NOVEL LYOPHILIZED FBS FOR CLINICAL IMMUNE MONITORING ASSAYS

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Background Interferon gamma (IFNγ) ELISpot and FluoroSpot are widely used immune monitoring assays to detect functional cell responses in clinical studies. Recognized for their importance in vaccine development studies to quantitate immune responses, these assays have more recently risen to the forefront in cell and gene therapy as well as cancer immunotherapy fields where responses against cancer neoantigens are not easily detectable above assay background.

Methods Here, we test a new class of fetal bovine serum (FBS), CultraPure FBS, in *ex vivo* ELISpot and FluoroSpot assays and in a cultured Fluorospot assay following *in vitro* expansion. Several CultraPure FBS lots that have been specially formulated through the process of lyophilization (lyo-FBS) were compared to liquid CultraPure FBS. We stimulated human PBMCs with peptide pools diluted in media supplemented with either liquid CultraPure FBS or lyo-FBS.

Results Equivalent cytokine production with negligible to no assay background were observed from all lots and FBS formats. Moreover, all lots and FBS formats showed lot-to-lot consistency and 90-day refrigerated (4°C) stability in both *ex-vivo* direct and *in vitro* cultured assays. In addition, we present here a FluoroSpot method using lyo-FBS supplemented media, cytokines, and peptide pools which supports the expansion of low frequency antigen-specific T cells, mimicking the low frequency seen with cancer neoantigens. Our results demonstrate the presence of Granzyme B, Interferon gamma (IFNγ), and Tumor Necrosis Factor (TNF) production by antigen-specific polyfunctional T cells following a 9-day culture using media supplemented with lyo-FBS.

Conclusions These results represent a new best practice and demonstrate the performance characteristics of a new lyophilized format of FBS which should improve the resolution of signal from noise in ELISpot and FluoroSpot immune monitoring in clinical studies.

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