In addition, cell viability remained very high 5 days after transfection with FectoPRO®, reaching comparable levels as non-transfected cells (Fig. 2). Maintained high cell viability allows consistent protein production, leading to the extremely high protein yields observed.

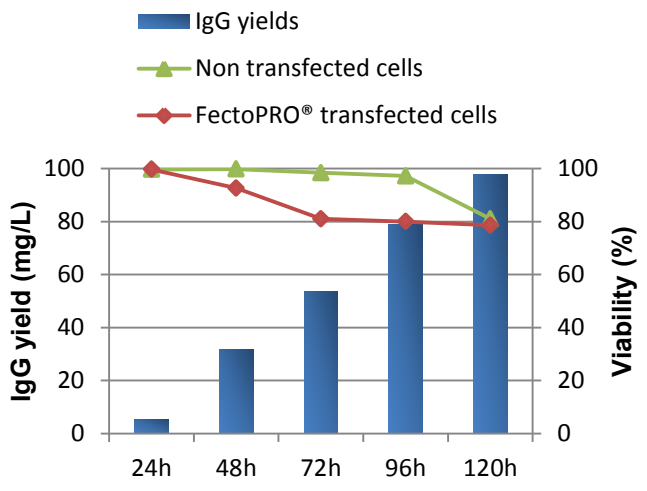

Fig 2: FectoPRO® leads to high cell viability in suspension CHO cells. FreeStyle™ CHO cells were transfected with FectoPRO® (0.8 µg DNA/ml). Protein yields were measured using fortéBIO™ and cell viability was analyzed using Trypan Blue.

Material and Methods

Transfection

CHO cells, cultured in Gibco® FreeStyle™ CHO Expression Medium (Life Technologies™), were transfected with several transfection reagents including FectoPRO®, by using vectors coding for the heavy (HC) and the light (LC) chain of a recombinant mouse IgG (ProteoGenix's pATX1-HC and pATX1-LC plasmids) and a proprietary booster plasmid.
For transfection with FectoPRO®, a total of **0.8 µg** of **DNA** was used per ml of cell culture, with a **1:1.5 (µg to µl) DNA to FectoPRO® ratio**. Previously optimized conditions were used for other reagents (FreeStyle™ MAX and PEI). Transfected cells were analyzed 1 to 5 days post-transfection.

IgG quantification

Protein quantification was performed using an Octet® RED96 system (fortéBIO®) according to manufacturer's instruction by using protein G Biosensors (Dip and Read™ Protein G (ProG) Biosensors, fortéBIO®). Transfection analysis was done at several time-points and the quantity of IgG in each sample was calculated by the integrated Octet® RED96 software.

Qualitative analysis of IgG production

Samples collected at each time point were loaded on 8% non-reducing PAGE after a semi-purification procedure. Briefly, 100 µl of culture medium was incubated with 10 µl of protein G sepharose for 30 min with agitation at room temperature. Protein G resin was then harvested, mixed with one volume of Laemmli sample buffer, and loaded on PAGE.

Determination of cell viability

Cell viability was determined by dye exclusion method with trypan blue reagent. Briefly, cells were mixed at 9:1 ratio with 0.4% trypan blue solution. Cell density and viability was determined with a disposable hemocytometer.

Altogether these data show a real advantage of using FectoPRO® for protein and antibody production with TGE in mammalian cells compared to commonly used technologies. FectoPRO leads to unmatched protein and antibody production yields, while maintaining high cell viability during the whole production process. ProteoGenix is proud to use this state-of-the-art technology from Polyplus-transfection to meet its customers' timelines and quality requirements.