## APPENDIX 1

***Colorimetric ELISA assay for G-CSF quantification***

This ELISA assay uses 96-well plates pre-coated with an anti-G-CSF monoclonal antibody. FFs and control were diluted 5 times in phosphate buffered saline. The same control (recombinant human G-CSF from Chinese Hamster Ovary) was used for each plate for inter-assay evaluation and the samples were measured in duplicate. A standard curve was prepared for a detection range between 0 and 182.9 pg/ml with the calibrator (recombinant Escherichia coli-derived human G-CSF). 100 µL of the standards 1-5 (0, 6.8, 20.3, 61, 182.9 pg/ml), the control and the samples were added in duplicate to the 96‑well plate. The plate was then sealed and incubated on a microplate shaker at 650 rpm at 25°C for 1 hr. The plate was washed three times with 350 µL of diluted wash buffer in a microplate washer. Then 100 µL of conjugate reagent (peroxidase conjugated mouse anti–G-CSF monoclonal antibody) was added to each well and the plate was incubated on a plate shaker at 25°C for 1 hr. The plate was washed as described previously and 100 µL of substrate solution (enhanced k-blue tetramethylbenzidine [TMB] substrate) was added to each well. The plate was incubated for 30 minutes at room temperature (18−25°C) in the dark. The colorimetric process was stopped by adding 100 µL of stop solution (1 molar hydrochloric acid) to each well. Absorbance was read at 450 nm within 5 minutes of adding the stop solution. A standard curve was generated from standards 1–5 using curve fitting software and a linear regression weight of 1/Y2.