Application note

Qkine lyophilized proteins are stable, bioactive and sterile after > 2 years storage in freezer

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Introduction

Qkine manufactures high-purity, animal origin-free growth factors, cytokines and other complex proteins for life science applications including stem cell and organoid culture. We've performed a series of tests to reassure our customers of the stability of our growth factors over an extended period at -80 °C and to show that there is no difference in quality between older and newer lots.

To assess stability, selected lyophilized proteins stored at -80 °C for > 2 years; activin A (Qk001), TGF-β1 PLUS™ (Qk010), Flt3L (Qk087), TGF-β3 (Qk054) and FGF-2 145 aa (Qk025) were tested. These were compared against the newest available batches (< 2 years old). These proteins were chosen to be representative of the Qkine catalog, covering different growth factor families and being lyophilized from different buffers.

This application note confirms that Qkine lyophilized proteins remain stable after > 2 years storage in the freezer.

Methods

Lyophilized proteins were stored at -80 °C for > 2 years. After reconstitution, these proteins were compared against proteins from the newest available batch (< 2 years old) stored under the same conditions. The bioactivity was tested using a luciferase reporter or proliferation bioassay, and 3 µg protein was resolved on a 15% w/v SDS-PAGE gel which was then stained with Coomassie Brilliant Blue R250.

Sterility testing was carried out by spiking proteins into TSB and FTM media and facilitating growth of potential microbial contaminants under aerobic (*Aspergillus brasiliensis & Bacillus subtilis*) and anaerobic (*Candida albicans & Escherichia coli*) conditions. Positive controls were grown under the same conditions for 2 weeks to demonstrate that these microbes can and will grow under the conditions provided.

Results

Qkine activin A (Qk001, figure 1), TGF-β1 PLUS™ (Qk010, figure 3), Flt3L (Qk087, figure 5), TGF-β3 (Qk054, figure 7) and FGF-2 145 aa (Qk025, figure 9) showed no loss in bioactivity after > 2 years at -80 °C. SDS-PAGE demonstrated that none of the proteins were degraded under these conditions (figures 2, 4, 6, 8, 10). Sterility testing showed no contamination with *Aspergillus brasiliensis, Bacillus subtilis, Candida albicans or Escherichia coli.*

Activin A (Qk001)

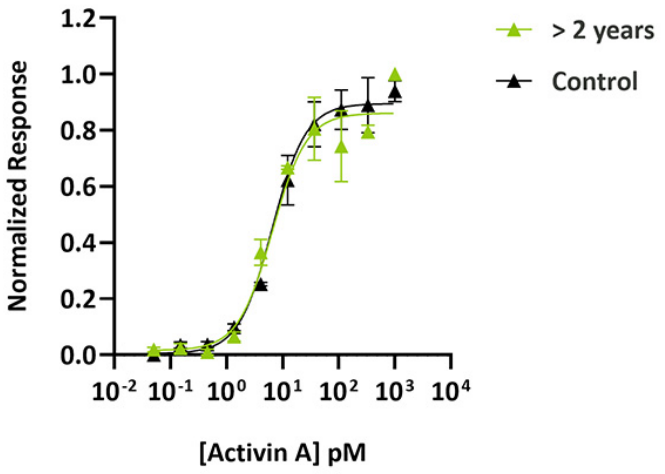

Figure 1: Activin A bioactivity remained stable for > 2 years
Bioactivity was determined using the activin-responsive firefly luciferase reporter assay in transiently transfected HEK293T cells. Cells were treated in triplicate with a serial dilution of activin A. Activin A stored at -80 °C for > 2 years (EC50 5.1 pM) showed no difference in bioactivity compared to the newest available batch (EC50 7.8 pM). Firefly luciferase activity was measured and normalized. Data from Qk001 lot #204545 (test) and #204771 (control).

TGF-β1 PLUS™ (Qk010)

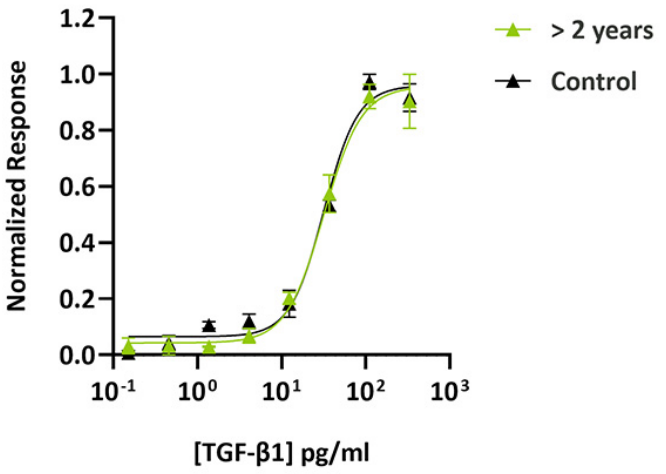

Figure 3. TGF-β1 PLUS™ bioactivity remained stable for > 2 years.
Bioactivity was determined using a TGF-β1-responsive (CAGA) firefly luciferase reporter in transiently transfected HEK293T cells. Cells were treated in triplicate with a serial dilution of TGF-β1. TGF-β1 stored at -80°C for > 2 years (EC50 29.0 pg/ml) showed no difference in bioactivity compared to the newest available batch (34.5 pg/ml). Firefly luciferase activity was measured and normalized. Data from Qk010 lot #204512 (test) and #204762 (control).

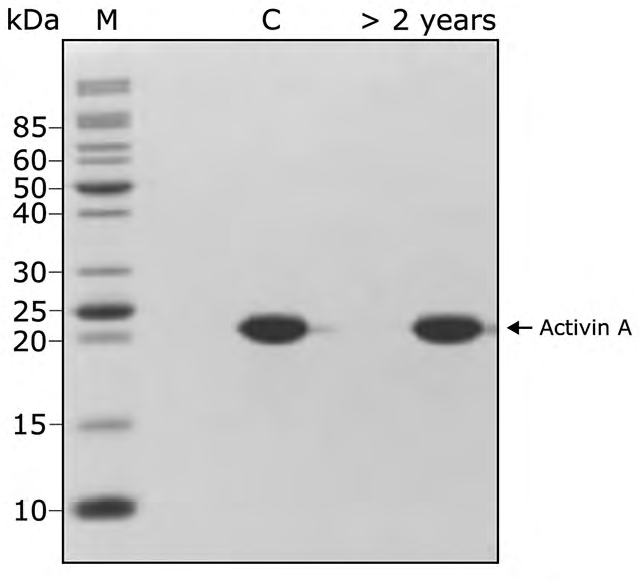

Figure 2. No degradation of activin A was visible after > 2 years.
Lyophilized protein was stored at -80 °C for > 2 years. 3 µg was resolved on a 15% w/v SDS-PAGE gel under non-reduced conditions and stained with Coomassie Brilliant Blue R250. Data from Qk001 lot #204545 (test) and #204771 (control).

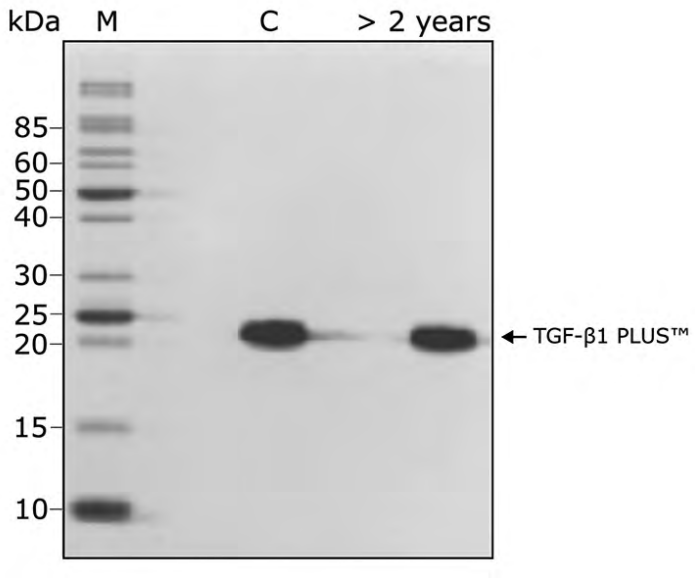

Figure 4. No degradation of TGF-β1 PLUS™ was visible after > 2 years.
TGF- β1 was stored at -80 °C for > 2 years. 3 µg was resolved on a 15% w/v SDS-PAGE gel under non-reduced conditions and stained with Coomassie Brilliant Blue R250. Data from Qk010 lot #204512 (test) and #204762 (control).

Flt3L (Qk087)

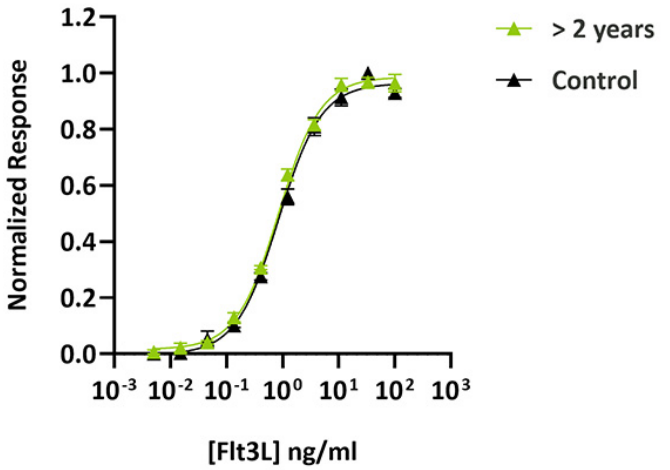

Figure 5. Flt3L bioactivity remained stable for > 2 years.
AML5 human myeloid leukemia cells were treated in triplicate with a serial dilution of Flt3L for 72 hours and proliferation measured using the CellTiter-Glo (Promega) assay. Flt3L stored at -80 °C for > 2 years (EC50 0.78 ng/ml) showed no difference in bioactivity compared to the newest available batch (EC50 0.94 ng/ml). Data from Qk087 #204566 (test) and #204633 (control).

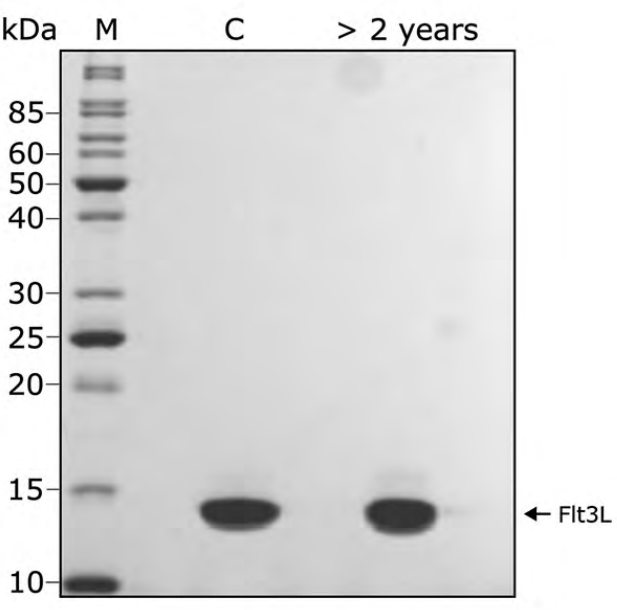

Figure 6. No degradation of Flt3L was visible after > 2 years.
Flt3L was stored at -80 °C for > 2 years. 3 µg was resolved on a 15% w/v SDS-PAGE gel under non-reduced conditions and stained with Coomassie Brilliant Blue R250. Data from Qk087 #204566 (test) and #204633 (control).

TGF-β3 (Qk054)

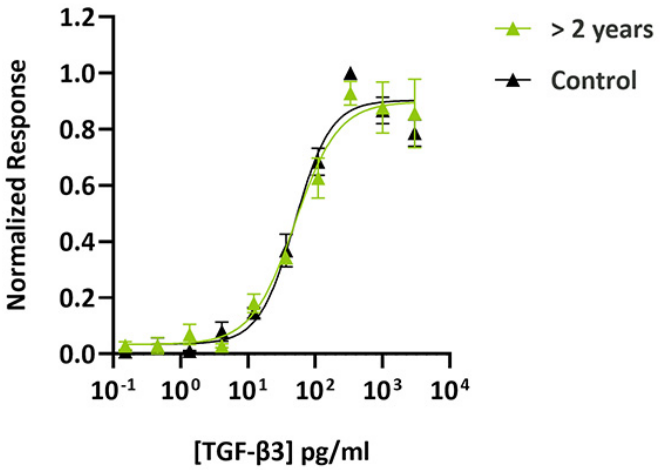

Figure 7. TGF-β3 bioactivity remained stable for > 2 years.
Bioactivity was determined using a TGF-β3 responsive firefly luciferase reporter in HEK293T cells. Cells were treated (in triplicate) with a serial dilution of TGF-β3 for 6 hours. TGF-β3 stored at -80 °C for > 2 years (EC50 54.75 pg/ml) showed no difference in bioactivity compared to the newest available batch (EC50 47.87 pg/ml). Firefly luciferase activity was measured and normalized to the control Renilla luciferase activity. Data from Qk054 batch #204489 (test) and #204616 (control).

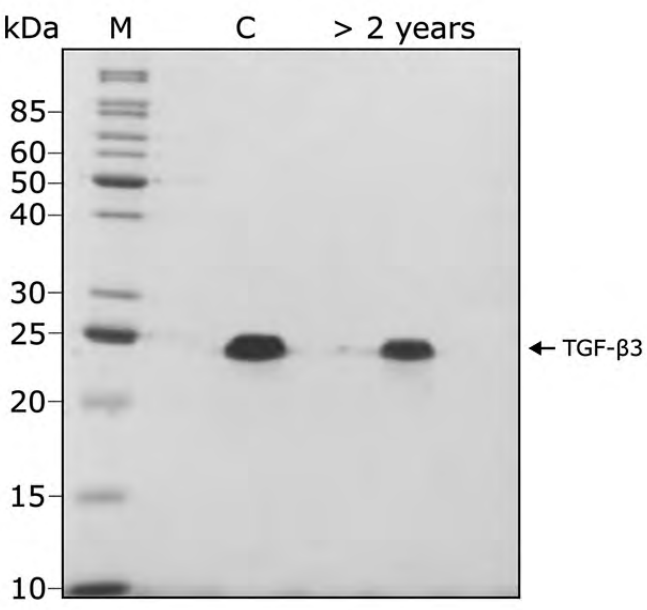

Figure 8. No degradation of TGF-β3 was visible after > 2 years.
TGF-β3 was stored at -80 °C for > 2 years. 3 µg was resolved on a 15% w/v SDS-PAGE gel under non-reduced conditions and stained with Coomassie Brilliant Blue R250. Data from Qk054 batch #204489 (test) and #204616 (control)

FGF-2 145 aa (Qk025)

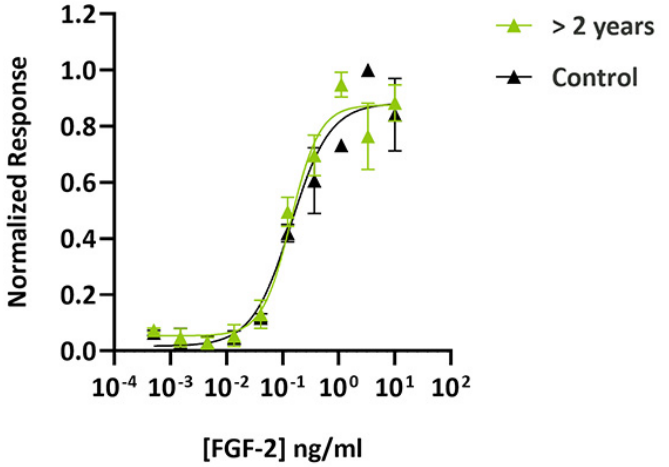

Figure 9. FGF-2 145 aa bioactivity remained stable for > 2 years. Bioactivity was determined using the Promega serum response element luciferase reporter assay in transiently transfected HEK293T cells. Cells were treated in triplicate with a serial dilution of FGF-2 145 aa for 6 hours. FGF-2 145 aa stored at -80°C for > 2 years (EC50 0.12 ng/ml) showed no difference in bioactivity compared to the newest available batch (EC50 0.19 ng/ml). Firefly luciferase activity was measured and normalized to the control Renilla luciferase activity. Data from Qk025 lot #204544 (test) and #204706 (control).

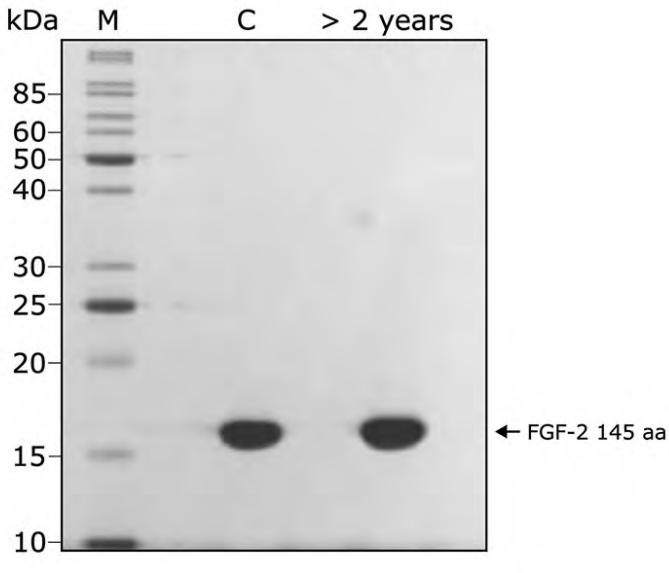

Figure 10. No degradation of FGF-2 145 aa was visible after > 2 years.
FGF-2 145 aa was stored at -80 °C for > 2 years. 3 µg was resolved on a 15% w/v SDS-PAGE gel under non-reduced conditions and stained with Coomassie Brilliant Blue R250. Data from Qk025 lot #204544 (test) and #204706 (control).

Conclusion

Qkine prides itself on the quality and bioactivity of its proteins. This testing demonstrated that there is no protein degradation or loss of activity over > 2 years of lyophilized protein storage at -80 °C, indicating that there is no effect of freezer storage over an extended period. Testing also showed the absence of common microbial contaminants.

Further information

Activin A (Qk001) – Recombinant activin A is our flagship product, animal origin-free (AOF), carrier protein-free, and tag-free to ensure its purity with exceptional lot-to-lot consistency. Activin A is used in many stem cell differentiation protocols, including endoderm lineage differentiation and further maturation into hepatocyte and pancreatic cells. Also available as cell therapy grade with extended quality testing and documentation – Qk001-CTG

TGF-β1 PLUSTM (Qk010) – Qkine recombinant TGF-β1 PLUS™ protein is the first entirely animal origin-free recombinant human TGF-β1 protein for highly reproducible results and compatible with chemically-defined stem cell media. Also available as cell therapy grade with extended quality testing and documentation – Qk010-CTG

Flt3L (Qk087) – Qkine highly bioactive Flt3L is suitable for the culture of reproducible and high-quality myeloid progenitors and dendritic cells. Also available as cell therapy grade with extended quality testing and documentation – Qk087-CTG

TGF-β3 (Qk054) – Highly pure and bioactive animal origin-free, Qkine TGF-β3 is used in human pluripotent stem cell maintenance media. Also available as cell therapy grade with extended quality testing and documentation – Qk054-CTG

FGF-2 145 aa (Qk025) – Our gold-standard and recommended FGF-2 protein for induced pluripotent stem cell (iPSC) and embryonic stem cell (ESC) maintenance, and iPSC and mesenchymal stem cell (MSC) proliferation and differentiation. Also available as cell therapy grade with extended quality testing and documentation – Qk025-CTG

For more information

Qkine growth factors are manufactured to the highest of quality standards and are free from animal-derived contaminants, delivering low endotoxicity and high purity. At Qkine, we are committed to raising the standards of growth factors, cytokines and related proteins to better support long-term and complex neural stem cell culture. We are a science-led team, please reach out with any questions or requests to support@qkine.com.

Please visit: **qkine.com**

To explore our high-purity, animal-free bioactive proteins visit qkine.com

Qkine products are for research use and ex vivo cell manufacturing use only

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