Application note

Stability of Qkine recombinant growth factors & cytokines in conditioned media

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Recombinant growth factors and cytokines are essential components in the successful culture of stem cells for translational research and the development of cellular agriculture. To preserve pluripotency, stem cells require exposure to consistent levels of specific growth factors and cytokines. The instability of recombinant protein media components can increase the need for frequent media changes to preserve cell conditions and this is costly.

Qkine strives to produce the most high-quality growth factors and cytokines for stem cell culture. Qkine ensures lot-to-lot consistency in bioactivity, high purity and animal origin-free production. This application note reviews the stability of Qkine growth factors under cell culture-like conditions to ensure maximum cost effectiveness and reproducibility in stem cell cultures.

Why is growth factor stability so important?

Stem cells are particularly sensitive to their cell culture environment; consistent exposure to essential growth factors is necessary to preserve pluripotency

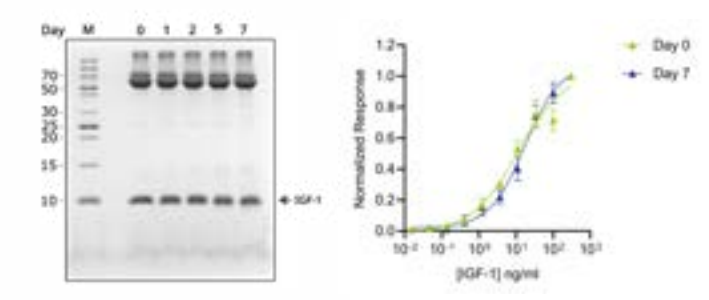
Reduced costs associated with cell culture through reducing the frequency of required media changes

Ensure reproducibility in phenotype of differentiated stem cells

Stability testing of Qkine growth factors

Conditioned media was collected from HEK293T cells cultured for 72 h in DMEM + 10% FBS and filtered. Qkine recombinant growth factors and cytokines IGF-1, GDNF, and HGF were reconstituted in phosphate buffered saline (PBS), BMP-4 and TGF-β1 in HCl reconstitution buffer at 1 mg/ml. Proteins were diluted to 0.5 mg/ml in PBS then diluted into the HEK293T conditioned media. Recombinant proteins in media were incubated at 37°C, 5% CO_2 and samples were taken at 0, 1, 2, 5 and 7 days. SDS-PAGE was used to assess stability at each time point and luciferase reporter or cell proliferation bioassays were used to assess bioactivity.

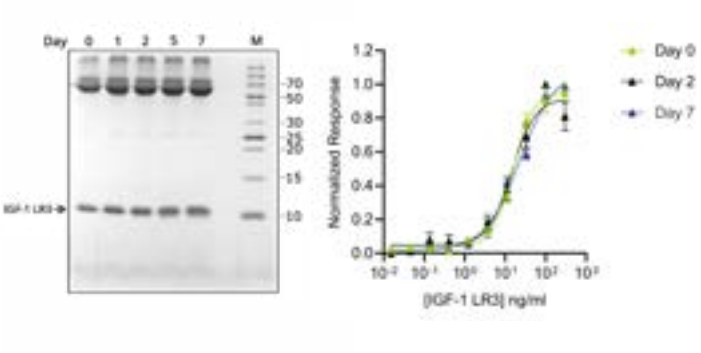
Qk047 Insulin-like growth factor 1 (IGF-1)



Stability and bioactivity of Qkine IGF-1 (Qk047). SDS-PAGE of recombinant IGF-1 showed that protein was not degraded after 7 days. Quantitative luciferase reporter assay demonstrated preserved bioactivity of IGF-1 after 7 days.

IGF-1 protein remained stable in conditioned media over the 7 days; it was as bioactive at day 0 (EC50 11.2 ng/ml) as it was at day 7 (EC50 17.2 ng/ml). IGF-1 is known to be relatively unstable in cell cultures, potentially due to the presence of metabolically active cells. However, in these experiments IGF-1 retained integrity and activity over 7 days.

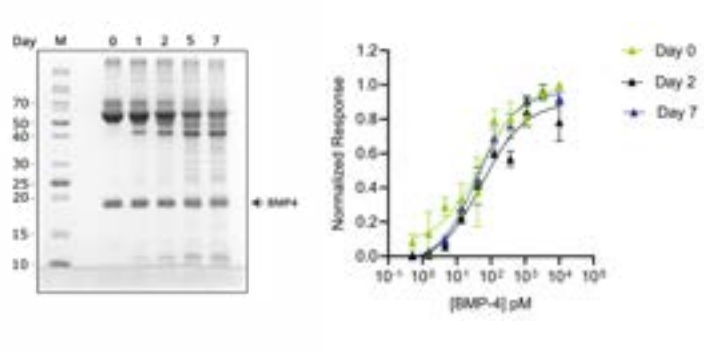
Qk041 Insulin-like growth factor 1 (IGF-1) LR3



Stability and bioactivity of Qkine IGF-1 LR3 (Qk041). SDS-PAGE of recombinant IGF-1 LR3; protein was not degraded after 7 days. Quantitative luciferase reporter assay demonstrated preserved bioactivity of IGF-1 LR3 after 2 and 7 days.

IGF-1 LR3 is a synthetic analog of IGF-1, modified to increase stability and reduce binding to inhibitory IGF-1 binding protein (IGFBPs). IGF-1 LR3 protein remained stable in conditioned media over the 7 days, it was bioactive at day 0 (EC50 15.5 ng/ml), day 2 (EC50 14.6 ng/ml) and day 7 (EC50 23 ng/ml).

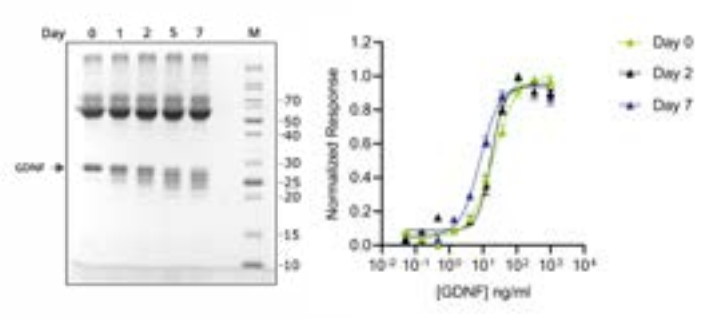
Qk038 Human bone morphogenetic protein 4 (BMP-4)



Stability and bioactivity of Qkine BMP-4 (Qk038). SDS-PAGE of recombinant BMP-4 protein showed no degradation after 7 days. Quantitative luciferase reporter assay demonstrated preserved bioactivity of BMP-4 after 2 and 7 days.

BMP-4 protein remained stable in conditioned media over the 7 days, it was bioactive at day 0 (EC50 51.2 pM), day 2 (EC50 53.6 pM) and day 7 (EC50 40 pM).

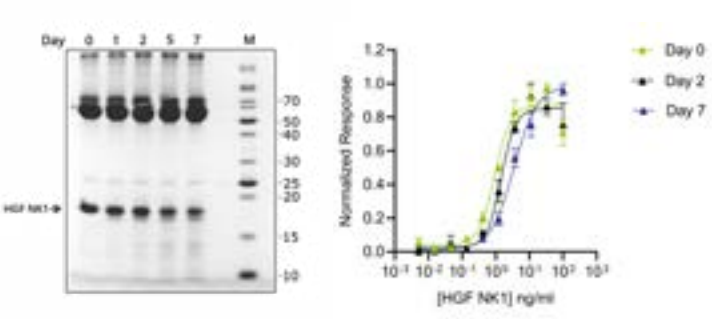
Qk051 Glial cell line-derived neurotrophic factor (GDNF)



Stability and bioactivity of Qkine GDNF (Qk051). SDS-PAGE showed recombinant GDNF degraded over the 7 days, however, quantitative proliferation assay showed preserved bioactivity of GDNF after 2 and 7 days.

Recombinant GDNF was progressively degraded over the 7 days in conditioned media. However, despite clear degradation occurring over the 7 days the bioactivity remained stable at day 0 (EC50 20.1 ng/ml), day 2 (EC50 17.3 ng/ml) and increased by day 7 (EC50 8.2 ng/ml). Interestingly, the portion of the GDNF which is responsible for activity appears to remain intact.

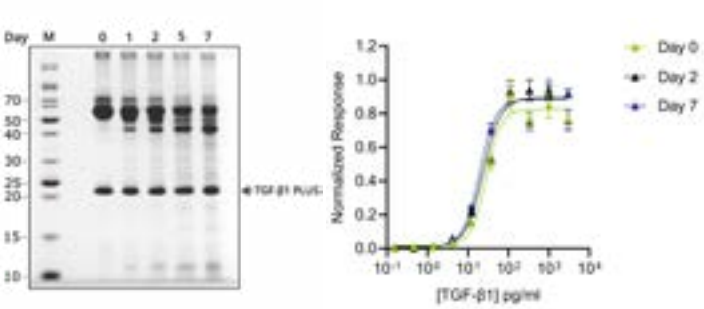
Qk013 Hepatocyte growth factor (HGF) NK1



Stability and bioactivity of Qkine HGF NK1 (Qk013). SDS-PAGE of recombinant HGF NK1 protein showed some degradation after 7 days. Quantitative luciferase reporter assay demonstrated bioactivity of HGF NK1 after 2 and 7 days.

HGF NK1 is a potent naturally occurring isoform of HGF. HGF NK1 protein showed some degradation over the 7 days. The bioactivity was also slightly decreased over time, however HGF NK1 was bioactive at day 0 (EC50 0.9 ng/ml), day 2 (EC50 1.4 ng/ml) and day 7 (EC50 3.5 ng/ml).

Qk010 Transforming growth factor-beta 1 (TGF-β1) PLUS



Stability and bioactivity of Qkine TGF-β1 PLUS (Qk010). SSDS-PAGE of recombinant TGF-β1 PLUS protein showed no degradation after 7 days. Quantitative luciferase reporter assay demonstrated preserved bioactivity of TGF-β1 PLUS after 2 and 7 days.

TGF-β1 protein remained stable in conditioned media over the 7 days, it was bioactive at day 0 (EC50 28.3 pg/ml), day 2 (EC50 23.8 pg/ml) and day 7 (EC50 19.5 pg/ml).

Stability of tested Qkine growth factors and cytokines

Cytokines IGF-1 (Qk047) and IGF-1 LR3 (Qk041) remained stable and bioactive after 7-day incubation in conditioned media. IGF-1 LR3 is modified to be more potent and stable than native IGF-1.

Growth factors BMP-4 (Qk038), HGF NK1 (Qk013), TGF-β1 (Qk010) were all found to be intact and bioactive after 7 days.

GDNF (Qk051) was the only growth factor which notably degraded progressively over 7 days. However, the bioactivity remained over this period.

However, the bioactivity remained over this period. Bioassays demonstrated that all tested growth factors maintained their bioactivity after 7 days in conditioned media, determining the thermostability of the proteins. During stem cell applications, cultured cells will be metabolically active, and this may have additional influence on growth factor stability, degradation and activity.

Stem cell culture models are developed for the study of disease and for cultivated meat applications. Stem cells require consistent exposure to essential growth factors to produce reproducible cell phenotypes. The stability of Qkine recombinant growth factors and cytokines in cell culture provide consistent cell culture conditions and reduce the need for daily media changes.

Further 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

For more information

Please contact our team: **customerservice@qkine.com** if you would like to discuss commercial or academic collaborations, supply agreements or any aspects of growth factor optimization and other products.

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