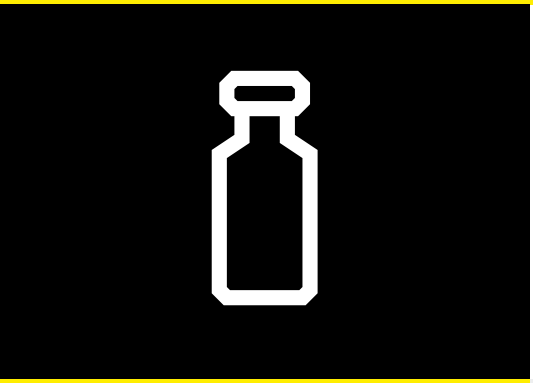
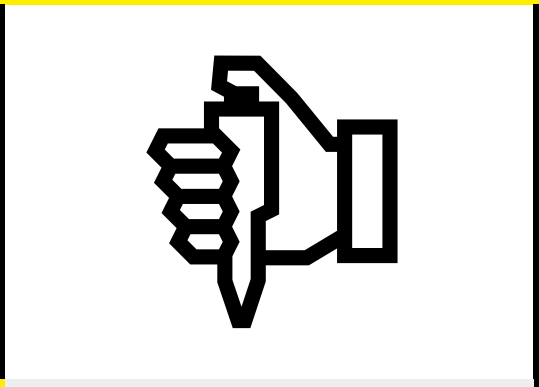
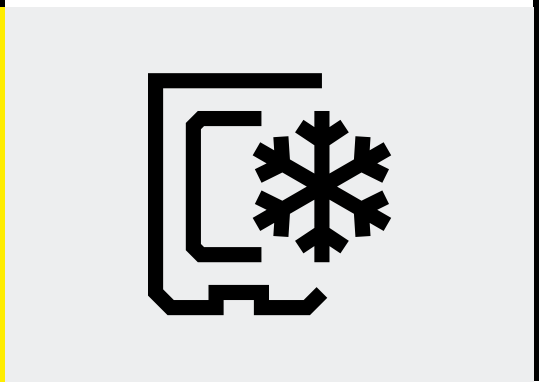
SARTORIUS

# Stability Studies

## Stability of CellGenix® GMP Cytokines after Reconstitution







### Technical Note

The quality, safety and efficacy of cell therapies are significantly influenced by the raw (ancillary) materials used in manufacturing. We prioritize safety and reliability to provide the highest quality products for clinical *ex vivo* cell processing..

## Executive Summary

Many of our customers would like to use our CellGenix® freeze-dried growth factors and cytokines at different time points after reconstitution. To support this flexibility we have put a validation procedure in place demonstrating product stability after reconstitution when handled under sterile conditions.

The following stability studies are included in our validation procedure:

- a freeze/thaw study comprising 4 cycles in a range from - 20 °C – + 4 °C
- a real time study at + 4 °C demonstrating a stability of 4 weeks
- a real time study at - 20 °C demonstrating a stability of 4 months

# All of our CellGenix® GMP cytokines expressed in *E. coli* have been validated:

| CellGenix® GMP cytokines | Active A | EGF | FGF-2 | Flt-3L | GM-CSF | IL-1ß | IL-2 | IL-3 | IL-4 | IL-6 | IL-7 | IL-10 | IL-15 | IL-21 | PDGF-BB | SCF | TNF-α | TPO |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Stability after 4 freeze/ thaw cycles in a range from - 20 °C to + 4 °C | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| Stability of 4 weeks at + 4 °C after reconstitution under sterile conditions | ■ | ■ | □ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| Stability of 4 months at - 20 °C after reconstitution under sterile conditions | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ |

Exemplary test results are shown in table 1 and figures 1 and 2 for the validation of our CellGenix® GMP rh IL-7 and CellGenix® GMP rh SCF.

## Test Conditions

All growth factors and cytokines, expressed in *E. coli,* have been reconstituted in sterile water or 0.2% acetic acid (IL-2 and EGF) to a concentration of 100 or 250 µg/ml. Cytokine activity was determined by performing a proliferation assay of the respective cytokine. Protein integrity was determined using size exclusion HPLC (SE-HPLC) or SDS-PAGE (10-20% gradient gel; Coomassie stain).

## Freeze/Thaw Study at - 20 °C to + 4 °C

Freeze-dried growth factors and cytokines were reconstituted under sterile conditions and subjected to 4 consecutive freeze/thaw cycles (16 h at - 20 °C/ 8 h at + 4 °C). Initial and final samples were analyzed for activity and protein integrity.

## Stability of Reconstituted Cytokines at + 4 °C

Freeze-dried growth factors and cytokines were reconstituted under sterile conditions and stored in the original container at + 4 °C for up to 4 weeks. Samples were analyzed weekly for activity and protein integrity.

## Stability of Reconstituted Cytokines at - 20 °C

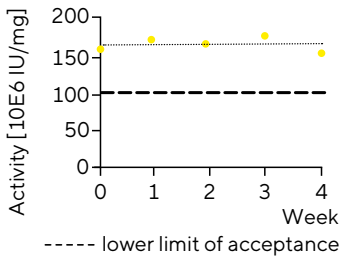
Freeze-dried growth factors and cytokines were reconstituted under sterile conditions. The reconstituted solution was stored in 60-80 µl aliquots in polypropylene cryogenic vials at - 20 °C for up to 4 months. Samples were analyzed monthly for activity and protein integrity.

**Table 1:** *Stability of reconstituted CellGenix® GMP rh IL-7 (#1010ii11) and CellGenix® GMP rh SCF (#1418LG22) evaluated at freeze/thaw conditions.*

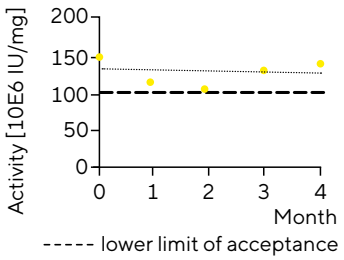
| Test Parameter | IL-7 | | SCF | |
|---|---|---|---|---|
| | Start | After 4 cycles | Start | After 4 cycles |
| Activity [10E6 IU/mg] | 135 | 136 | 0.9 | 0.9 |
| % Oligomers | < 0.5 | < 0.5 | < 0.5 | < 0.5 |

**Figure 1:** *Stability of reconstituted CellGenix® GMP rh IL-7 (#1010ii11) stored at + 4 °C for up to 4 weeks and at - 20 °C for up to 4 months. a/b) Activity was determined using IL-7 dependent IxNI2b cells. c/d) Formation of oligomers was determined using SE-HPLC.*

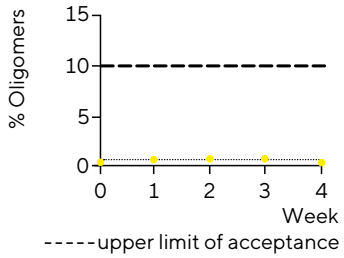
(A) **Activity IL-7 stored at + 4 °C**




(B) **Activity IL-7 stored at - 20 °C**




(C) **% Oligomers IL-7 stored at + 4 °C**




(D) **% Oligomers IL-7 stored at - 20 °C**

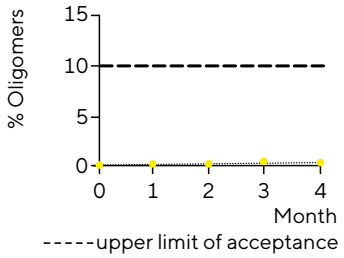
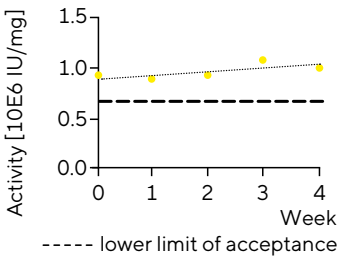
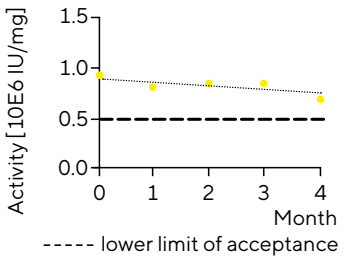



**Figure 2:** *Stability of reconstituted CellGenix® GMP rh SCF (#1418LG22) stored at + 4 °C for up to 4 weeks and at - 20 °C for up to 4 months. a/b) Activity was determined using SCF dependent TF-1 cells. c/d) Formation of oligomers was determined using SE-HPLC.*

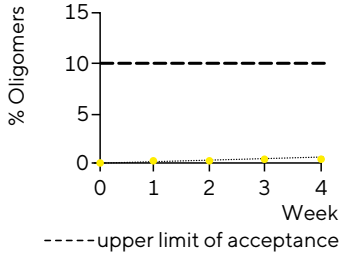
(A) **Activity SCF stored at + 4 °C**



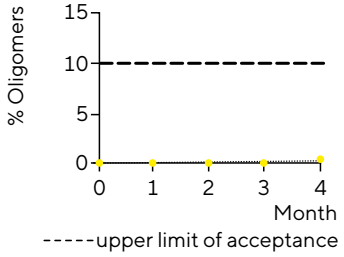

(B) **Activity SCF stored at - 20 °C**




(C) **% Oligomers SCF stored at + 4 °C**




(D) **% Oligomers SCF stored at - 20 °C**




## Conclusion

The product quality of our reconstituted CellGenix® GMP growth factors and cytokines is not impaired at either experimental condition. The results of our freeze/thaw study and real time studies at + 4 °C and - 20 °C demonstrate that the activity and integrity is maintained after reconstitution under the defined conditions.

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