TECHNOTE – STABILITY STUDIES

Stability of CellGenix® GMP Cytokines after Reconstitution

Many of our customers would like to use our CellGenix® freeze-dried 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 to +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

The large majority our CellGenix® GMP cytokine portfolio has been validated:

CellGenix® GMP cytokines	Activin A	EGF	FGF-2	Flt-3L	GM-CSF	IL-2	IL-3	IL-4	IL-6	IL-7	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	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v
Stability of 4 weeks at +4°C after reconstitution under sterile conditions	v	v		v	v	v	v	v	v	v	v	v	v	v	v	v
Stability of 4 months at -20°C after reconstitution under sterile conditions	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v

Stability studies for our remaining cytokines are still ongoing. We will publish an updated list on a regular basis. 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 cytokines have been reconstituted in sterile water or 0.2 % acetic acid (IL-2 and EGF) to a concentration of 100-250 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 cytokines were reconstituted under sterile conditions and subjected to 4 consecutive freeze/thaw cycles (16h at -20°C / 8h at +4°C). Initial and final samples were analyzed for activity and protein integrity.

Stability of Reconstituted Cytokines at +4°C

Freeze-dried 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 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.

Test Parameter	IL-7		SCF	
	Start	After 4 cycles	Start	After 4 cycles
Activity [10^6 IU/mg]	135	136	0.9	0.9
Oligomers [%]	< 0.5	< 0.5	< 0.5	< 0.5

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

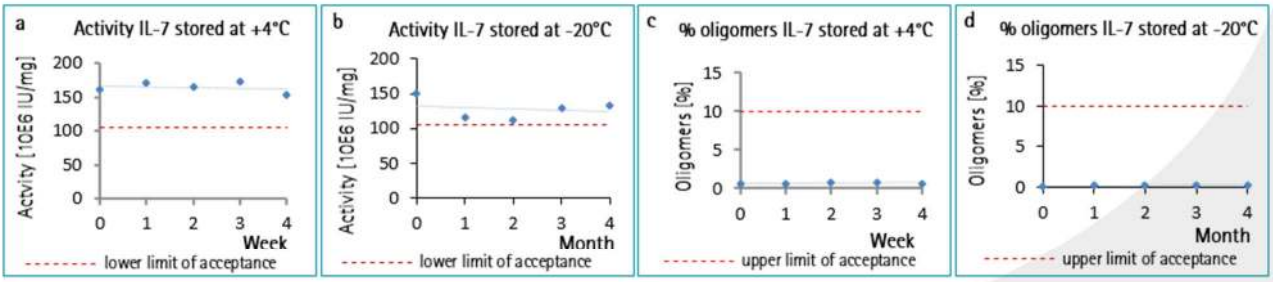

Fig 1: Stability of reconstituted CellGenix® GMP rh IL-7 (#1010ii11) stored at +4°C for up to 4 weeks and at -20°C for up to 4 months. a/b) Activity was determined using IL-7 dependent IxNI2b cells. c/d) Formation of oligomers was determined using SE-HPLC.

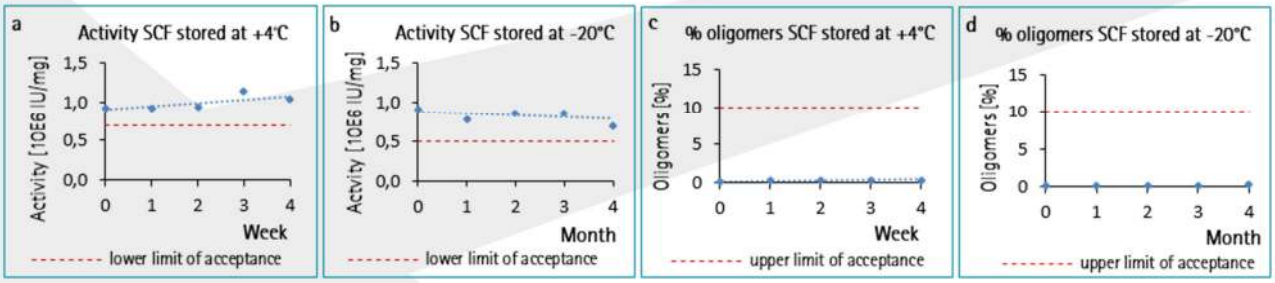

Fig 2: Stability of reconstituted CellGenix® GMP rh SCF (#1418LG22) stored at +4°C for up to 4 weeks and at -20°C for up to 4 months. a/b) Activity was determined using SCF dependent TF-1 cells. c/d) Formation of oligomers was determined using SE-HPLC.

Conclusion

The product quality of our reconstituted CellGenix® GMP 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.

Regulatory Excellence

CellGenix GMP products are based on three major quality standards:

- **Safety** - Safe and qualified raw materials in compliance with our animal-derived component-free and serum-free policy.
- **GMP Compliance** - Manufacturing and quality control following all applicable GMP guidelines to provide documented evidence of purity, potency, consistency and stability.
- **Regulatory Compliance & Support** – GMP products are manufactured, tested, released and distributed under an ISO 9001:2008 certified Quality Management System and allow for the safe use in accordance with USP Chapter <1043> and Ph. Eur. General Chapter 5.2.12. GMP cytokines are tested and released according to USP Chapter <92> as applicable.

We offer expert regulatory and technical support as well as FDA Drug Master Files for most of our products. Customized solutions can be provided to meet special compliance needs.

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