

## Supplementary Document – Quality Attributes

Key and critical quality attributes of the cellular API and of the finished HAC-based therapeutic product are presented in tabular form (i.e., [Tables SQA1](#) and [SQA2](#), respectively) in this supplementary document “Quality Attributes”. API, active pharmaceutical ingredient; HAC, human articular chondrocyte.

**Table SQA1.** Established cryopreserved cellular API (i.e., cultured HACs) quality attributes, which were specified as KQA or as CQA. API, active pharmaceutical ingredient; CQA, critical quality attribute; EU, endotoxin units; HAC, human articular chondrocyte; KQA, key quality attribute.

API Quality Attributes	Attribute Type	Requirements for an API Lot (Cumulative)
• Cellular API Identity	CQA	Appropriate cellular morphology and behaviour in two-dimensional culture, appropriate increase in chondrogenic gene expression in three-dimensional culture, appropriate specific gene expression markers.
• Cellular API Purity	KQA	Appropriate cellular morphology and behaviour in two-dimensional culture, appropriate specific gene expression markers.
• Cellular API Function	CQA	Appropriate increase in chondrogenic gene expression in three-dimensional culture.
• Sterility (bacteria and fungi)	CQA	Absence of detection for specified and non-specified contaminants or values of detection < to specified thresholds.
• Sterility (mycoplasma)	CQA	Absence of detection for specified and non-specified contaminants.
• Acceptable Endotoxin Level	CQA	Endotoxin level < 0.2 EU/mL.
• Cellular Viability Maintenance	CQA	Cellular viability maintenance throughout storage resulting in cellular viability of > 85% upon initiation from storage.
• Cellular Proliferation Capacity Maintenance	CQA	Possibility of in vitro monolayer adherence and expansion for $\geq 3$ passages.
• Number of Cells/API Container	KQA	Specified number of cells/container $\pm 20\%$ .
• API Identification	CQA	Correct labelling of API packaging materials.
• Appropriate API Storage	CQA	Cryogenic API storage at temperatures constantly < $-145\text{ }^{\circ}\text{C}$ .
• Appropriate API Validity	CQA	Use of the API within the validated API validity period.

**Table SQA2.** Established HAC-based injectable therapeutic product quality attributes, which were specified as KQA or as CQA. API, active pharmaceutical ingredient; CQA, critical quality attribute; EU, endotoxin units; HAC, human articular chondrocyte; KQA, key quality attribute.

Finished Product Quality Attributes	Attribute Type	Requirements for a Finished Product Lot (Cumulative)
• Formulated API Identity	CQA	Appropriate traceability for formulation of the correct API in the finished product.
• Sterility (bacteria and fungi)	CQA	Absence of detection for specified and non-specified contaminants or values of detection < to specified thresholds.
• Sterility (mycoplasma)	CQA	Absence of detection for specified and non-specified contaminants.
• Acceptable Endotoxin Level	CQA	Endotoxin level < 0.2 EU/mL.
• Cellular Viability Maintenance	CQA	Cellular viability > 75% in the finished product during the whole validated product validity period.
• Finished Product Tonicity & pH	KQA	Appropriate product tonicity and pH for the considered administration route.
• Total Cell Dose	KQA	Specified number of cells/product dose $\pm$ 20%.
• Product Quantity	KQA	Appropriate product quantity at the time of reconciliation with the medical prescription.
• Product Administration System	CQA	Appropriate administration system for the planned surgical operation, as prescribed.
• Product Identification	CQA	Correct labelling of product packaging materials and product primary container.
• Appropriate Product Storage	CQA	Ambient temperature storage of the finished product.
• Appropriate Product Validity	CQA	Use of the finished product within the validated product validity period.