Gamma Irradiation of Human Platelet Lysate: Validation of Efficacy for Pathogen Reduction and Assessment of Impacts on hPL Performance

Gamma irradiation is one of the most widely employed methods for pathogen reduction and commercial gamma sterilization facilities are easily accessible. The whole system for manufacturing gamma irradiated fetal bovine serum (FBS) has been well-established, including dose range, dose mapping, frozen condition, as well as validation of pathogen reduction. Nevertheless, many research articles have addressed the optimal conditions for utilizing gamma irradiation in human plasma and blood components. With these comprehensive references, we previously assessed the feasibility of using gamma irradiation to obtain pathogen-reduced human platelet lysate (hPL) and reported low impacts on the potency for cell expansion.

In this study, we validated the efficacy of gamma irradiation for virus inactivation. Four model viruses (BVDV, Reo3, HSV1, MMV) were chosen, per ICH/EMA guidelines, to represent a range of viruses with different genome, structure, size, and sensitivity to various chemical and physical agents. The virus spiked hPLs were gamma irradiated and the mean values of viral titers showed more than 4 log10 reduction across all model viruses. The results demonstrated gamma irradiation is an effective viral reduction procedure for hPL.

To assess the impacts of gamma irradiation on the long-term stability of hPL performance, we analyzed UltraGRO™ GI series up to one year after gamma irradiation. The results showed growth factors still retained comparable levels to the non-irradiated hPLs. Mesenchymal stromal cells (MSC) cultured with gamma irradiated hPLs for more than three passages showed similar profiles as with the corresponding non-irradiated hPLs in respect of growth rate, morphology, immunophenotype, trilineage differentiation potency, and immunosuppressive property.

COMPARISON OF PATHOGEN REDUCTION TREATMENT (PRT) FETAL BOVINE SERUM (FBS) VS. ULTRAGRO™ GI SERIES VIRAL CLEARANCE VALIDATION

PRT FBS	Vs.	UltraGRO™ GI Series
0.22µm	Sterile filtration	0.22µm
<-10° C	Finished products storage	-20° C
Frozen	Transportation to irradiation plant	Frozen on dry ice
Gamma	Irradiation	Gamma
Cobalt-60	Radiation source	Cobalt-60
5-60 kGy (viral inactivation study) 25-40 kGy (typically employed for commercial products)	Dosage	25-40 kGy
Sealed containers	Physical state	Sealed bottles
Dry ice	Temperature control	Dry ice
Frozen	Transportation to supplier storage	Frozen on dry ice

Virus Category	RNA Enveloped	RNA Non-Enveloped	DNA Enveloped	DNA Non-Enveloped
Model for	HCV, HIV	HAV	CMV, EBV, HBV	B19
Virus	BVDV	Reo3	HSV1	MMV
Family	Flavi	Reo	Herpes	Parvo
Genome	ssRNA	dsRNA	dsDNA	ssDNA
Size (nm)	40-60	60-80	120-200	18-24
Resistance	Low	Med-High	Medium	Very High
UltraGRO™-PURE GI	> 5.42	> 4.40	> 4.51	4.55
UltraGRO™ -Advanced GI	> 5.54	> 4.27	> 4.50	4.46

Reference: AventaCell 2019 ISCT Poster

MSCs Culture with UltraGRO™-PURE GI GMP

Gamma Irradiated/ Viral Inactivate
Xeno-free, Safe, Consistent, Cost effective cell culture

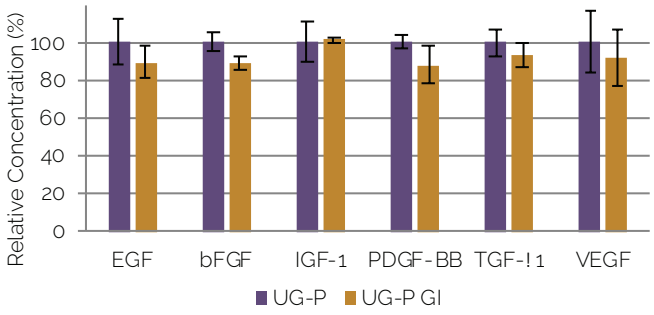
BENEFITS OF ULTRAGRO™ PURE GI

- **US FDA DMF # 34284**
- **JAPAN PMDA Certificate**
- **Ph. Eur. General Chapter 5.2.12.4 Compliance**
- UltraGRO™-PURE GI supplements for producing clinical grade cells
- Gamma irradiation has been accepted by regulatory agencies as a validated PRT
- Comparable cell culture performance maintained
- Viral inactivated products without loss of potency

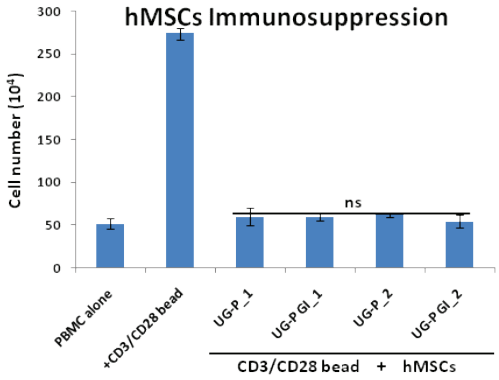
AventaCell BioMedical has adopted a state-of-the-art gamma irradiation process, as a pathogen reduction treatment (PRT), for viral inactivation to create an UltraGRO™-PURE GI (UG-P GI) product. UG-P GI offers minimized pathogen contamination risk while preserving potent cell culture performance with human mesenchymal stem cells (hMSCs), human immune cells and other applicable cell types for clinical applications.

Marker %	Cell type	CD73	CD90	CD105	CD34	CD45	CD11b	CD79a	HLA-DR
UG-P GI	AD-MSC	99.97	99.88	95.33	0.34	0.40	0.78	0.37	1.65
	UC-MSC	95.51	99.98	99.09	0.80	0.31	1.08	1.11	1.97
	BM-MSC	99.94	99.50	99.95	0.93	0.15	0.15	0.34	1.45

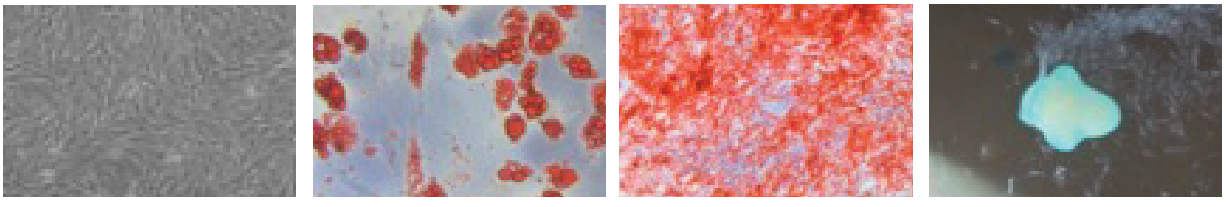
Immuno-phenotypical characterization of human MSCs. Human MSCs derived from adipose tissue (AD), umbilical cord matrix (UC), bone marrow (BM) cultured in UltraGRO™-PURE GI for 5 passages displayed characteristic expression of MSC surface markers.



Growth factors retained comparable cytokine levels after receiving gamma irradiation



MSCs retained immunomodulation potency



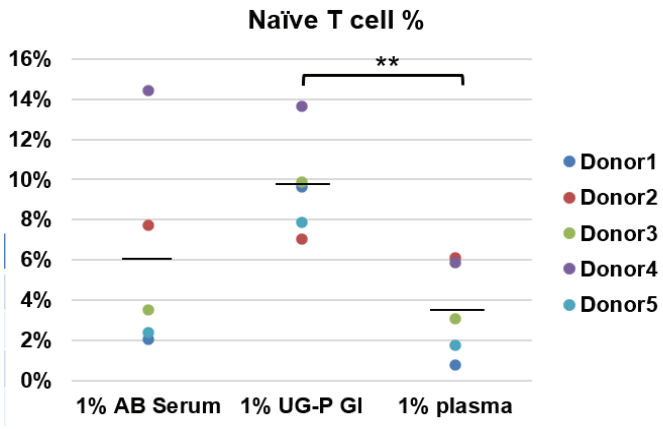
AD MSC | Adipo-genesis | Osteo-genesis | Chondro-genesis

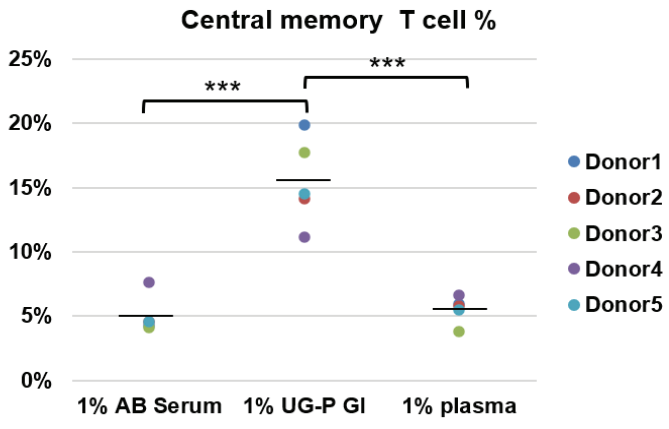
Human adipose tissue derived MSCs retain tri-lineage differentiation capability after cultured in UltraGRO™-PURE GI supplemented medium for three passages

Reference: AventaCell UltraGRO™-PURE GI MSC Factsheet

Immune Cell Culture with UltraGRO™-PURE GI GMP

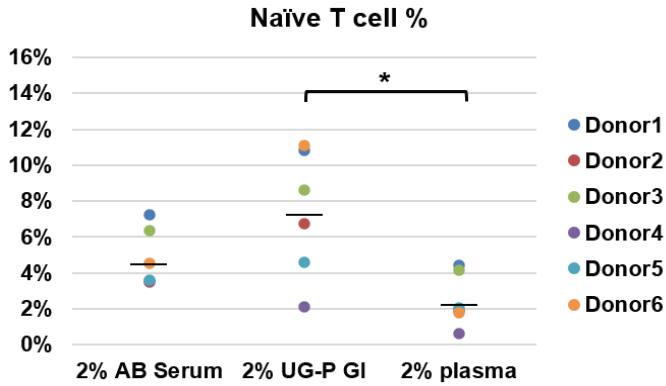
Xeno-free, Gamma Irradiate and Viral Inactivated hPL for Therapeutic T cell Activation

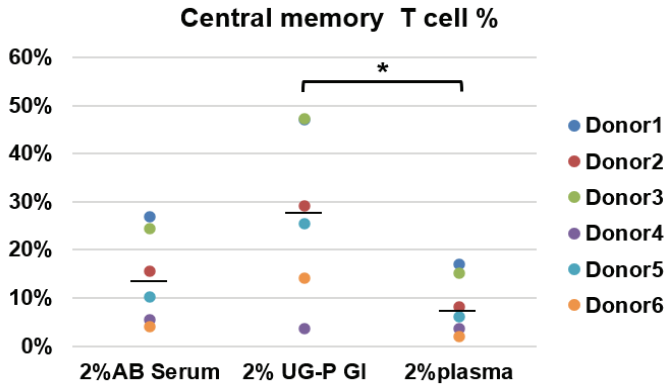




Ave (n = 5)	1% AB serum	1% UG-P GI	1% plasma
Expansion	1321 folds	2610 folds	1708 folds
T cell %	86	82	83
Naïve T %	6.0	9.6	2.5
CM T %	5.0	15.5	6

PBMCs were collected from 5 healthy donors, and T cells were activated by applying a commercial kit from supplier A, followed by the manufacturer's protocol to compare the induction performance with AB serum, UG-P GI, and auto-plasma. The results showed greater T cells with higher population of Naïve and central memory T cells could be obtained by introducing UG-P GI.





Ave (n = 6)	2% AB serum	2% UG-P GI	2% plasma
Expansion	998 folds	2336 folds	1527 folds
T cell %	80	85	78
Naïve T %	4.8	7.4	2.5
CM T %	14.4	27.8	8.6

Product Number	Product	Bottle Size (mL)
HPCHXCGLI05	UltraGRO™-PURE GI GMP	50
HPCHXCGLI50		500

PBMCs were collected from 6 healthy donors, and T cells were activated by applying a commercial kit from supplier B, followed by the manufacturer's protocol to compare the induction performance with AB serum, UG-P GI, and auto-plasma. The results showed greater T cells with higher population of Naïve and central memory T cells could be obtained by introducing UG-P GI.

Reference: AventaCell UltraGRO-PURE GI Immune cell Factsheet