

## **AMNIOTIC MEMBRANE PROCESSING: MAINTAINING CHARACTERISTICS OF SOURCE TISSUE WHILE MAXIMIZING NORMALIZATION, QUALITY AND SAFETY.**

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### **INTRODUCTION**

Research on the therapeutic potential of perinatal birth tissue in a clinical setting has increased exponentially as clinicians, scientists, researchers and manufacturers continue to innovate and utilize this tissue with patients for its regenerative properties. In order to effectively measure outcomes, several factors must be considered. Birth tissue is dynamic and varies widely from one donation to the next. Standardization (reduction of variations from each lot in birth tissue) of a final product is critical to clinical utility. Without proper processing techniques, it is not possible to address this variance and ensure product quality, product safety and product consistency.

Predictive Biotech's human cell and tissue product (HCT/P) AmnioCyte Plus™ is derived from the amniotic membrane from donated, full-term birth tissue. Equipped with state-of-the-art analytical equipment to ensure the safety and viability of its allografts, Predictive's laboratory and proprietary processes maintain the tissue's biological components, yielding a normalized dose. AmnioCyte Plus is comprised of cytokines, growth factors, exosomes and scaffolding proteins.

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## BACKGROUND

### Acellular Amniotic Membrane

The fetal membranes consist of two layers: the outer chorion which is vascular and in contact with the uterine wall, and the amnion (amniotic membrane) which is avascular, lies inner to the chorion and is in contact with amniotic fluid. The amniotic membrane's basal surface lies on top of the chorion, the outer most layer of the placental membrane.<sup>1</sup> The amniotic membrane and the chorion are not bonded and remain separable even after delivery.<sup>2</sup>

Human amniotic membrane is a thin transparent membrane that is mostly comprised of collagen, laminin, and fibronectin,<sup>3</sup> its composition includes:

- Epithelium
- Basement membrane composed of: collagen types III, IV, V; laminin, fibronectin, nidogen
- Compact layer composed of: collagen types I=I, III, V, VI; fibronectin
- Fibroblast layer composed of: collagen types I, III, VI; nidogen, laminin, fibronectin
- Intermediate (spongy) layer composed of: collagen types I, III, IV; proteoglycans

### Function of Amniotic Membrane

The amniotic membrane is biological structure devoid of nerves, muscles or lymph vessels. It receives nutrients and oxygen from the chorionic fluid and transfers it across the membrane to the fetal surface vessels.<sup>4</sup> As it protects the growing fetus from the pressures of surrounding structures, it also facilitates metabolic functions, such as the transport of water and soluble materials, and produces important biological factors, such as cytokines and growth factors.<sup>5</sup>

### AmnioCyte Plus Product Description

AmnioCyte Plus is processed to preserve the structural integrity of the amniotic membrane and meets the definition of minimally manipulated as described in the U.S. Food and Drug Administration's (FDA) published "Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use", for structural tissue (2017). The processing of AmnioCyte Plus does not alter the original tissue's function as a protective tissue, providing physical support and cushioning.

AmnioCyte Plus is processed mechanically to maintain the tissue's biological components, its composition has been analyzed by an in-depth human cytokine array and an enzyme-linked immunosorbent assay (ELISA) array.

## CYTOKINES

Cytokines are a broad group of small cellular proteins involved in biological function. Birth tissue contains a rich and diverse population of cytokines, underscoring the dynamic nature of the tissue. These often-overlooked proteins are critical tissue components that must be accounted for and maintained in the manufacturing process of donated tissue. Predictive products were analyzed by RayBiotech in a Quantibody® analysis, an array-based multiplex ELISA system for simultaneous quantitative measurement of various cytokines, growth factors, proteases, soluble receptors and other proteins. (Figure 1)

Test	Description	AmnioCyte Plus avg pg/mL	Test	Description	AmnioCyte Plus avg pg/mL	Test	Description	AmnioCyte Plus avg pg/mL
CYT5	Ferritin	328,296.3	CYT6	Galectin-3	5,106.5	CYT8	IGFBP-5	1,645.8
CYT7	Fetuin A	273,161.9	CYT4	Resistin	4,764.7	CYT4	Siglec-5	1,644.3
CYT8	DPPIV	65,701.7	CYT8	IL-5 Ra	4,646.6	CYT10	Galectin-9	1,633.1
CYT5	Adiponectin	63,751.6	CYT5	IGF-1R	4,573.0	INF3	IL-1ra	881.8
CYT6	APRIL	61,546.3	REC1	PECAM-1	2,114.3	INF3	TNF RI	868.7
CYT9	Angiotensinogen	59,503.0	CYT10	Pentraxin 3	4,505.5	CYT9	EMMPRIN	1,593.8
INF3	TIMP-2	6,723.6	CYT5	MMP-9	4,483.4	CYT6	FAP	1,533.6
CYT7	TSP-1	55,739.1	CYT9	FLRG	4,412.1	CYT6	Nephrilysin	1,471.1
CYT9	Periostin	39,157.6	CYT10	ADAM8	4,261.5	CYT6	DcR3	1,469.9
CYT10	CD84	38,858.4	CYT4	Galectin-7	4,085.8	CYT6	WIF-1	1,444.4
CYT5	ANGPTL4	36,877.6	CYT10	CD48	4,060.9	CYT8	MMP-7	1,382.9
CYT4	VEGF R1	26,987.3	CYT9	Persephin	3,964.0	CYT7	WISP-1	1,374.7
CYT4	PAI-1	24,160.0	CYT9	Tie-1	3,784.1	CYT4	E-Cadherin	1,345.8
CYT9	Follistatin-like 1	22,311.2	CYT9	B7-H1	3,749.6	CYT6	IGF-2R	1,338.3
CYT5	MMP-2	14,746.3	CYT5	CA125	3,744.8	REC1	MICB	717.6
CYT7	VE-Cadherin	14,646.4	REC1	ALCAM	1,670.0	CYT4	IL-13 R2	1,289.6
CYT9	Thrombospondin-5	13,237.4	CYT6	Thrombomodulin	3,636.3	CYT4	ICAM-2	1,254.7
CYT7	Albumin	12,679.5	CYT7	Dkk-3	3,535.4	CYT8	CHI3L1	1,235.2
CYT5	hCGb	12,458.3	CYT7	Decorin	3,231.8	CYT7	TACI	1,205.2
CYT7	CD163	11,841.0	CYT8	Cathepsin B	3,143.8	CYT9	IL-17E	1,204.1
CYT9	L1CAM-2	10,970.3	CYT4	DKK-1	3,056.9	CYT8	PSMA	1,177.2
CYT9	DNAM-1	10,868.1	REC1	Trappin-2	1,572.2	CYT10	Galectin-2	1,173.9
CYT9	LRP-6	10,646.5	CYT6	sFRP-3	2,967.7	CYT7	Clusterin	1,172.6
CYT10	Pref-1	10,552.1	CYT7	TRANCE	2,831.6	CYT7	ANG-4	1,171.7
CYT9	ADAMTS13	9,159.8	REC1	CD14	1,493.7	CYT10	DR3	1,140.3
CYT7	Furin	8,912.8	CYT4	gp130	2,828.0	CYT5	NCAM-1	1,123.8
INF3	ICAM-1	3,891.9	CYT9	Granulysin	2,669.4	REC1	uPAR	647.2
CYT5	Adipsin	8,744.1	CYT6	Cathepsin L	2,649.1	REC1	RAGE	645.3
CYT9	CD6	8,674.3	CYT10	CEACAM-5	2,611.2	CYT5	OSM	1,115.8
CYT4	DAN	8,557.6	CYT10	SP-D	2,610.1	REC1	Lipocalin-2	637.8
CYT10	CD155	8,395.7	CYT5	MMP-1	2,608.5	CYT7	RANK	1,093.6
CYT7	Syndecan-1	8,164.1	CYT4	Fcg RIIBC	2,583.2	CYT5	AFP	1,078.3
INF3	TIMP-1	3,475.2	CYT10	ICOS	2,402.5	CYT9	Aggrecan	1,067.4
CYT7	RBP4	8,131.9	CYT10	CD58	2,400.2	CYT7	LAG-3	1,009.7
CYT6	Chemerin	7,962.2	CYT9	CNTF	2,333.4	CYT5	CA15-3	1,002.2
CYT5	Nidogen-1	7,708.3	CYT7	ACE-2	2,329.2	CYT5	MMP-13	991.5
CYT7	CA19-9	7,676.8	CYT5	CRP	2,328.5	REC1	VCAM-1	592.8
CYT5	NSE	7,658.1	CYT5	Procalcitonin	2,305.9	CYT10	ULBP-1	972.7
CYT4	Angiostatin	7,618.5	CYT8	Leptin R	2,298.4	CYT9	BMPR-II	912.9
CYT9	LRIG3	7,476.4	CYT7	AMIICA	2,013.3	CYT5	Thyroglobulin	837.6
CYT6	CD97	7,205.5	CYT10	B7-H3	1,934.9	CYT6	IFNab R2	818.3
CYT8	biG-H3	7,075.2	CYT9	Nectin-4	1,890.4	CYT6	Legumain	755.9
CYT8	IL-10 Ra	6,457.2	CYT10	Cadherin-4	1,884.6	CYT8	IL-1 F10	747.5
CYT5	Prolactin	6,344.5	CYT7	DLL1	1,874.3	CYT4	FAS L	737.9
CYT10	Siglec-10	6,296.7	CYT8	IL-1 F7	1,824.1	CYT8	SIGIRR	735.0
CYT9	BMPR-IA	6,058.5	CYT6	CD200	1,807.9	CYT6	HGF R	709.2
CYT9	Fractalkine	6,023.8	CYT7	IL-17B R	1,793.7	REC1	LYVE-1	507.7
CYT6	Transferrin	5,737.8	CYT10	Cystatin B	1,724.8	CYT9	ULBP-2	700.2
CYT6	Serpin A4	5,533.8	CYT4	IL-13 R1	1,704.6	CYT6	C5a	695.6
CYT10	Desmoglein 2	5,377.1	CYT7	CXCL14	1,665.5	CYT6	IL-1 R6	689.0

**Figure 1:** Representative list of 150 of the 171 present in the cytokine array characterization in AmnioCyte Plus, as analyzed by RayBiotech (average pg/ml).

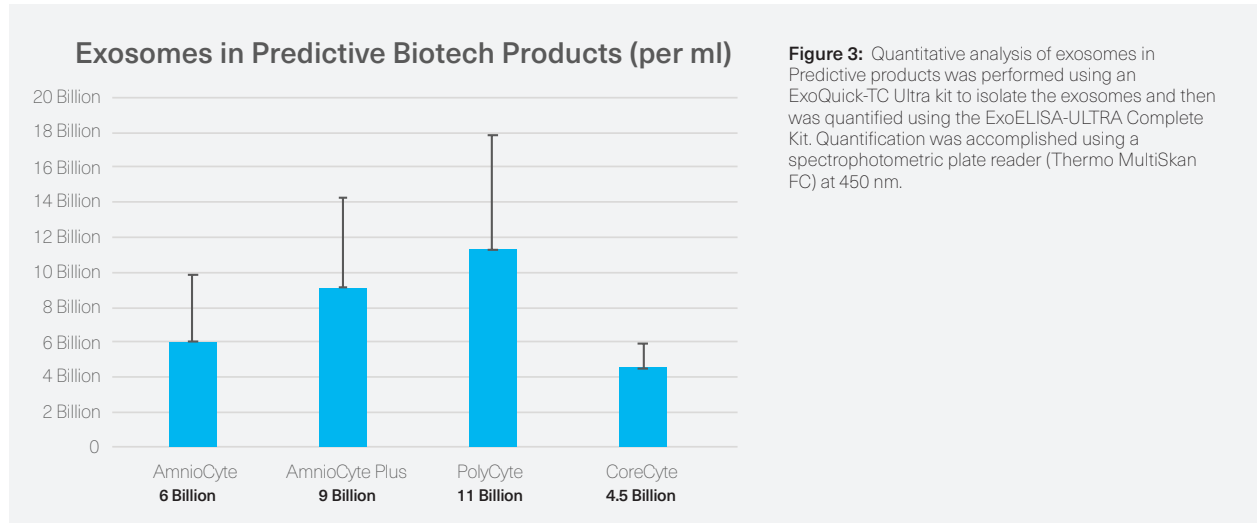
Each component of birth tissue has a specific function in the development of a fetus. Those tissue components produce cytokines specific to the gestational function they serve. (Figure 2)

Description	AmnioCyte (avg. pg/mL)	AmnioCyte Plus (avg. pg/mL)	PolyCyte (avg. pg/mL)	CoreCyte (avg. pg/mL)
Fetuin A	138,263	273,162	53,053	345,099
IGFBP-3	67,986	35,281	1,146	2,436
hCGb	55,037	12,458	2,552	0
TSP-1	36,437	55,739	85,932	3,514
IGFBP-6	35,915	17,096	895	1,770
CA15-3	30,540	1,002	837	1,303
SP-D	30,400	2,610	274	49
PAI-1	29,445	24,160	25,018	2,065
DPPIV	24,894	65,702	151,431	19,661
CA125	23,974	3,745	22,723	3,657
APRIL	23,065	61,546	9,745	12,353
Pref-1	20,068	10,552	24,729	484
IGFBP-4	18,401	10,555	2,450	7
FLRG	17,176	4,412	26,625	148
Angiotensinogen	15,421	59,503	199	74,688
Adiponectin	14,970	63,752	294,627	4,766
OPN	14,954	11,487	5	328
VEGF R1	14,530	26,987	24,610	1,012
IGFBP-1	13,371	14,114	5,572	62
CA19-9	11,737	7,677	132,302	617
Thrombospondin-5	11,668	13,237	14	140
Albumin	10,816	12,679	28,969	10,807
TNF RII	10,169	379	825	1,168
Adipsin	9,974	8,744	21,394	2,053
MIF	9,407	28,702	34,833	4,915
MMP-7	8,862	1,383	1,088	0
uPAR	8,527	647	315	69
ICAM-1	8,318	3,892	4,850	6,413
Follistatin-like 1	8,121	22,311	237	9,548
TIMP-2	8,045	6,724	6,690	951
CD97	3,305	7,206	58,961	651
Chemerin	1,858	7,962	48,162	497
Leptin R	0	2,298	30,908	12
Thrombospondin-2	621	673	16,477	150
blG-H3	7,340	7,075	16,358	3,534
Activin A	68	268	16,245	145
RBP4	7,058	8,132	14,664	8,259
NSE	391	7,658	13,811	4,726
Serpin A4	7,762	5,534	13,310	6,573
Prolactin	3,670	6,345	13,233	244
MCSF R	5,388	2,772	10,079	4,121
Ferritin	265	328,296	601,435	232,582
Periostin	5,239	39,158	81,724	784
CD84	0	38,858	1,008	2,995
ANGPTL4	1,966	36,878	63,272	21,414
MMP-2	4,924	14,746	12,261	3,551
VE-Cadherin	165	14,646	1,498	1,652
PF4	2,202	14,370	38,007	955
CD163	3,238	11,841	31,969	89
L1CAM-2	0	10,970	3,057	61,822
DNAM-1	0	10,868	2,210	258
LRP-6	0	10,646	3,883	483

**Figure 2:** Quantitative analysis of 52 cytokines present in varying concentrations across Predictive's products. The 52 cytokines are a combination of the 30 highest concentrations for each product

## EXOSOMES

Predictive quantified the exosome presence of its products as a part of the product's tissue profile using a standard ELISA (Figure 3). Post-thaw analysis confirmed that AmnioCyte Plus contains naturally occurring exosomes from birth tissue and quantified the value to be approximately 9.0 billion exosomes per 1 ml of product. Exosomes are membrane-bound extracellular vesicles that are present in all bodily fluids under both normal and pathophysiological conditions.<sup>6</sup> Exosomes are produced in the endosomal compartment of most eukaryotic cells. An ELISA is a plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies and hormones.



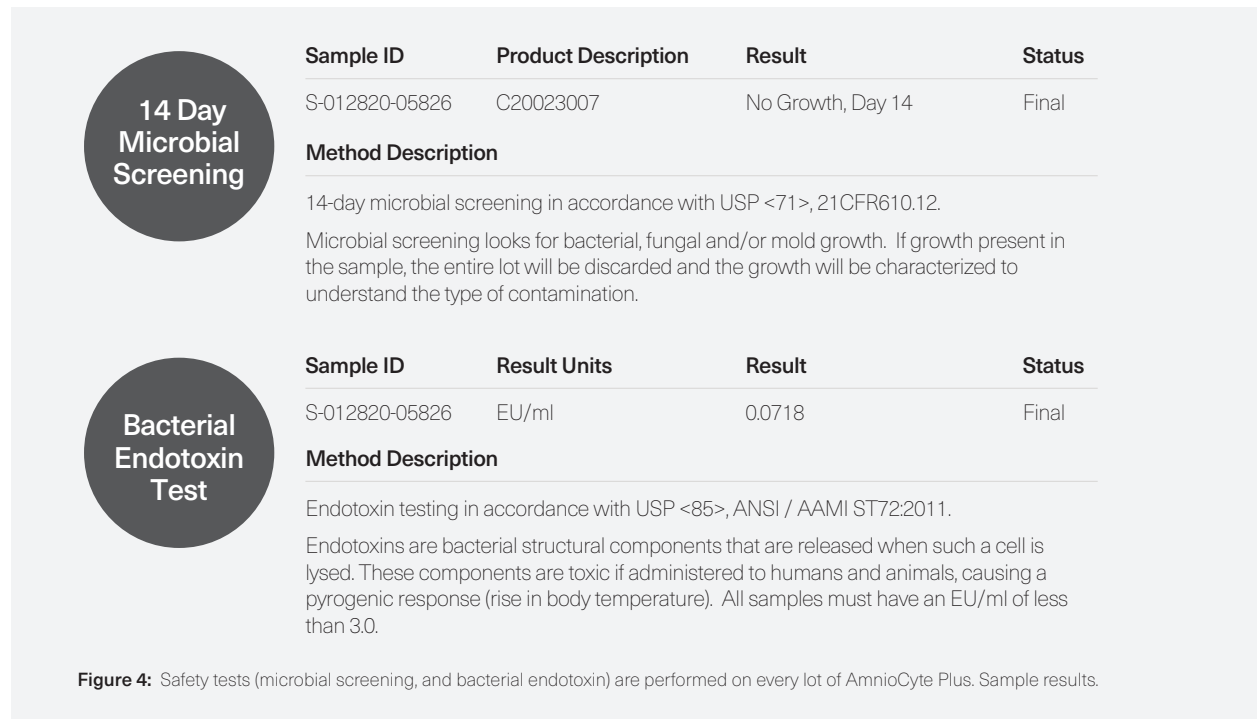
**Figure 3:** Quantitative analysis of exosomes in Predictive products was performed using an ExoQuick-TC Ultra kit to isolate the exosomes and then was quantified using the ExoELISA-ULTRA Complete Kit. Quantification was accomplished using a spectrophotometric plate reader (Thermo MultiSkan FC) at 450 nm.

## PRODUCT SAFETY

AmnioCyte Plus is manufactured under Current Good Manufacturing Practice (cGMP), Current Good Tissue Practice (cGTP) and is ISO 13485 accredited. AmnioCyte Plus is derived from amniotic membranes obtained from donors after normal, full-term pregnancies. Each donor is carefully screened, with comprehensive medical and social histories of donors collected for review by a medical doctor. All tissue is procured, processed and tested in accordance with FDA requirements to minimize potential risks of disease transmission to recipients. Infectious disease testing is performed at a certified laboratory in accordance with the Clinical Laboratory Improvement Amendments of 1988 (CLIA) and 42 CFR part 493.

## PRODUCT SAFETY PROCESS

Birth tissue is shipped to Predictive's lab within 72 hours of birth. Upon arrival, Predictive's quality assurance team performs inspections to verify that tissue viability has been properly maintained during transport. All tissue is processed in Predictive's ISO 7 cleanroom under ISO 5 biological safety cabinets. Once produced, two (2) samples of each lot of AmnioCyte Plus are sent for safety testing. All remaining product is placed into cryogenic quarantine (release pending third-party validation and medical director approval). The two (2) samples are sent to a third-party laboratory for serology and bacterial endotoxin testing (Figure 4).



**Figure 4:** Safety tests (microbial screening, and bacterial endotoxin) are performed on every lot of AmnioCyte Plus. Sample results.

Predictive's medical director reviews donor's medical records and blood test, and deems the donor eligible or ineligible to donate tissue.

<b>DONOR ELIGIBILITY SCREEN</b>		
<b>Test Description</b>	<b>Standards</b>	<b>Status</b>
HIV-1/HIV-2 Antibody	Non-Reactive	Final
HIV-1/HIV-2 NAT	Non-Reactive	Final
Hepatitis B Surface and Core Antibody	Non-Reactive	Final
Hepatitis B NAT	Non-Reactive	Final
Hepatitis C Antibody	Non-Reactive	Final
Hepatitis C NAT	Non-Reactive	Final
Syphilis	Non-Reactive	Final
West Nile Virus	Non-Reactive	Final
Cytomegalovirus IgM	Not Detected	Final
Microbial Screening – Bacterial	No Growth (USP 71)	Final
Microbial Screening – Fungal	No Growth (USP 71)	Final
Bacterial Endotoxin (BET)	≤3 EU/ml	Final

**Method Description**

Infectious disease testing is performed at a certified laboratory in accordance with the Clinical Laboratory Improvement Amendments of 1988 (CLIA) and 42 CFR part 493.

**Figure 5:** Donor eligibility screening panel.

Should tissue fail any safety test (microbial screening or endotoxin) or the donor eligibility requirements, the entire lot of AmnioCyte Plus will fail quarantine and be discarded.

Once third-party analysis is received and approved, Predictive's quality assurance team conducts a final review of the entire process. If review is approved, the individual lot of product will be released from quarantine. AmnioCyte Plus is shipped overnight on dry ice to keep product at appropriate temperature to maintain product viability.

## CONCLUSION

AmnioCyte Plus is processed at Predictive Biotech's state-of-the-art laboratory, where each step is monitored carefully for quality assurance. AmnioCyte Plus is comprised of cytokines, growth factors, exosomes and scaffolding proteins as validated by third-party labs and internal measures.

The investment in research, processes, equipment, facilities and third-party testing has provided Predictive the ability to develop the safest and most consistent amniotic membrane allograft available in the market. With over 100,000 allografts shipped, Predictive's processing, safety, and normalization standards have established AmnioCyte Plus as a market leading product.

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