

# Certificate of Analysis

<b>Product Description:</b>	AccuCell® Characterized Human Peripheral Blood Mononuclear Cells (PBMCs), Purified
<b>Product Number:</b>	93000-10M
<b>Lot Number:</b>	2010113198
<b>Storage Temperature:</b>	≤ -150°C, Vapor Phase Liquid Nitrogen
<b>Preservative:</b>	DMSO
<b>Vial Fill Volume:</b>	1.5 mL
<b>Manufacture Date:</b>	15-JUL-2015
<b>Expiration Date:</b>	15-JUL-2018
<b>Intended Use:</b>	For Research Use Only. Not for Use in Diagnostic or Therapeutic Procedures.

**Caution:** Use Universal Precautions for handling AccuCell® Human PBMC and other human specimens. Do not pipette by mouth. Avoid direct inhalation of the solution and use with adequate ventilation. Do not smoke, eat, or drink, in areas where specimens are being handled. Dispose of this product as appropriate for biohazardous material.

PARAMETER	SPECIFICATION	RESULT
Viable Cell Count Per Vial Post-Thaw/Post-Wash:*	≥ 1.0e7 viable cells	1.4e7 viable cells
Cell Viability Post-Thaw/Post-Wash: Viability based on assessment using propidium iodide.*	≥ 80%	88%
Cell Proliferation Index (PI): The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; thiazolyl blue) assay measures cell proliferation in response to PHA mitogen. An input of 200,000 cells/well are plated in a 96 well plate plus or minus PHA. The amount of proliferation is detected using MTT solution after 96 hours of incubation. The Proliferation Index is the ratio of cell supernatant absorbance at 570 nm in wells when stimulated by PHA divided by cell absorbance without PHA.	≥ 2.0	3.2
ELISpot Reactivity: IFN-γ responses against PHA mitogen and CMV and CEF peptide pools measured in an ELISpot assay. Results are represented as Spot Forming Cells (SFC) per well based on an input of 200,000 cells/well.	CEF Pool CMV Pool PHA	0 SFC 0 SFC 457 SFC
HLA Class I HLA Class II	A B C DR DQ	A*01:01 A*24:02 B*15:17 B*35:02 C*04:01 C*07:01 DRB1*11:04/11:104 DRB1*13:02 DRB3*02:02/02:26/02:28/02:29N DRB3*03:01

PARAMETER	SPECIFICATION	RESULT
		DQB1*03:01/03:22 DQB1*06:04/06:39 Bw4 is present Bw6 is present
Donor Current Medications	Any	Flomax, Simvastatin, Tamsulosin, Ranitidine, Omeprazole, Terazosin, Ibuprofen
Donor Age	18-75 years	59 years
Donor Height	centimeters	178 cm
Donor Weight	kilograms	91 Kg
Donor Gender	Male/Female	Male
Donor Ethnicity	Any	Caucasian
Anticoagulant	ACD, Citrate, CPD, CPDA, CPDA-1, CPDA-2 or Na-Hep	Citrate
HIV-1**	Negative	Negative
HIV-2**	Negative	Negative
HBV**	Negative	Negative
HCV**	Negative	Negative

\* Typical lot release data stated in COA for total viable cells and viability % are based on the thawing procedure described in the Product Insert.

\*\* The donor unit used for preparation of this product was collected from an FDA regulated center and tested with FDA approved test kits for the listed viruses. The donor was presumed to be healthy at the time of donation; however, there can be no certainty that the donor was disease free.

Prepared by: 

Date: 11/3/2015

QA Review by: 

Date: 11-03-2015

## Antibody-dependent Cell-mediated Cytotoxicity (ADCC) Assay Results

The ADCC of PBMCs produced by Precision are measured by incubating target cells (SK-BR-3, breast adenocarcinoma cells) with an antibody specific to a target cell marker (Herceptin biosimilar antibody), followed by addition of the PBMCs under test. An effector:target cell ratio of 25:1 is used and a control with a non-specific antibody (ET901, a fully human IgG<sub>1</sub> isotype) is used to measure non-specific lysis. PBMCs are thawed and allowed to rest overnight at 37°C prior to testing to reduce the assay background. The lysis of the target cells is measured after 16 hours of culture using the CytoTox 96™ Non-Radioactive Cytotoxicity Assay manufactured by Promega (Catalogue number G1780). This assay quantitatively measures lactate dehydrogenase (LDH), a stable cytosolic enzyme that is released upon cell lysis. Released LDH in culture supernatants is measured with a coupled enzymatic assay, which results in the conversion of a tetrazolium salt into a red formazan product, with the amount of color formed being proportional to the number of lysed cells. Controls are included in the assay for non-specific killing, viability, and background issues.

The percent specific lysis is calculated according to the LDH kit manufacturer's recommendations. Values are first corrected for background by subtracting the average of absorbance values of the culture medium alone from all absorbance values of Experimental, Target cell spontaneous LDH release, and Effector cell Spontaneous LDH release. If after subtraction of background a value is negative it is rounded up to zero. These corrected values are then used in the following calculation:

$$\% \text{ Cytotoxicity} = \frac{\text{Experimental} - \text{Effector Spontaneous} - \text{Target Spontaneous}}{\text{Target Maximum} - \text{Target Spontaneous}} \times 100$$

PBMCs were classified as Positive in the ADCC test if specific lysis was greater than the mean + 3 standard deviations of non-specific LDH release. Results are summarized in Table 1 for the lots listed.



**Table 1: Results of Antibody Dependent Cell Cytotoxicity for the PBMC lots listed (Cells tested at an effector/target ratio of 25:1)**

PBMC lot	ADCC Classification	Specific Lysis		Non-specific Lysis		The mean % non-specific lysis plus 3x standard deviations.
		Mean (%)	SD (%)	Mean (%)	SD (%)	
13224	Negative	9.5	1.24	-2.53	4.05	9.62
13226	Negative	4.18	0.19	-3.54	5.67	13.47
13227	Positive	14.69	0.29	-3	0.33	-2.01
13223	Positive	21.93	0.29	-0.67	0.05	-0.52
13194	Positive	18.22	2.36	1.45	2.74	9.67
13219	Positive	16.55	3.71	4.86	0.15	5.31
13220	Positive	31.08	5.72	-0.49	2.24	6.23
13213	Weak Positive	8.85	1.89	-4.83	1.16	-1.35
13193	Weak Positive	12.39	5.22	0.71	3.57	11.42
13228	Positive	25.03	0.87	0.15	1.96	6.03
13229	Positive	7.9	8.13	-2.21	1.3	1.69
13230	Positive	15.74	0.96	-6.55	2.39	0.62
13232	Positive	10.75	1.4	0.636	1.96	6.516
13233	Weak Positive	2.55	1.31	0.76	0.37	1.87
13222	Weak Positive	4.53	2.38	-3.9	0.47	-2.49
13198	Negative	-3.77	3.88	-2.74	2.06	3.44
13242	Positive	11.28	3.57	-1.87	0.13	-1.48
13252	Indeterminate	0	0	0	0	
13238	Positive	12.36	5.02	3	1.66	7.98
13241	Positive	27.58	1.54	2.43	0.28	3.27
13253	Negative	0.92	3.24	-5.95	2.67	2.06
13251	Weak Positive	8.84	3.44	-8.5	1.82	-3.04


Note that Lots 13213, 13193, 13233, 13222 and 13251 are designated as weak positives because the mean specific lysis value is greater than the mean of the non-specific lysis plus 3 standard deviations. However, we would not recommend use of these lots if a positive result in an ADCC assay is required.



**Table 2: Results of Antibody Dependent Cell Cytotoxicity for the PBMC lots listed (Cells tested at an effector/target ratio of 10:1, where data is not available for a ratio of 25:1)**


PBMC lot	ADCC Classification	Specific Lysis		Non-specific Lysis		The mean % non-specific lysis plus 3x standard deviations.
		Mean (%)	SD (%)	Mean (%)	SD (%)	
13216	Positive	12.94	0.04	1.26	2	7.26
13217	Positive	43.44	5.28	-4.6	1.83	0.89

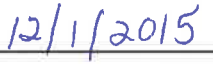
**Prepared by:**

  
\_\_\_\_\_  
Deborah Phippard, Vice President, Research

  
\_\_\_\_\_  
Date

**Approved by:**

  
\_\_\_\_\_  
Carolyn Anderson, Senior Program Manager

  
\_\_\_\_\_  
Date