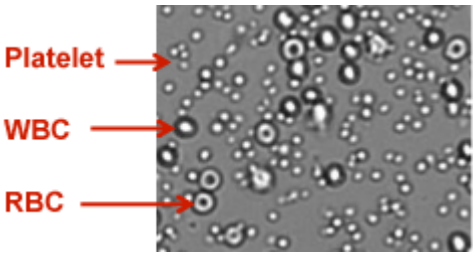
PBMC 细胞的精确计数和活性分析

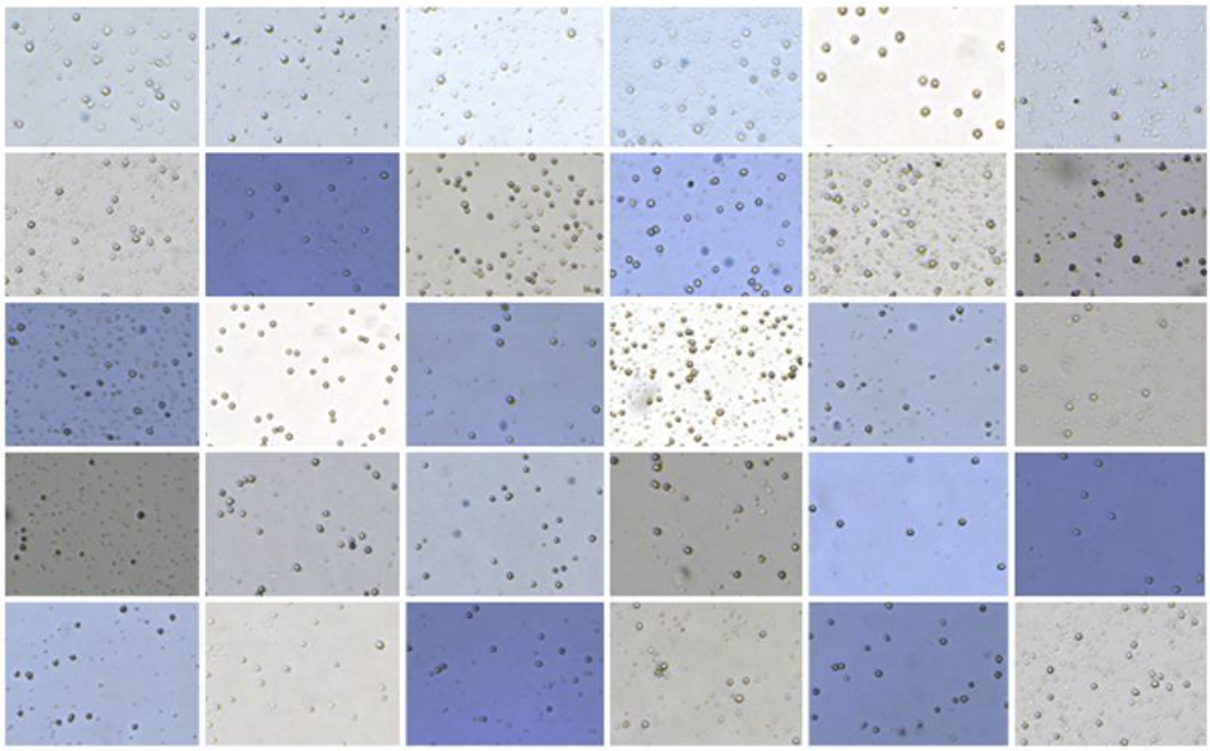
一、 PBMC 细胞计数的现状

1. 目前，绝大多数的科研工作者仍在使用血球计数板在显微镜下进行手工计数 PBMCs；
2. 但是显微镜下由于白细胞和红细胞大小非常接近，不能完全区分白细胞和红细胞；
3. 通过红细胞的双凹形形态鉴别红细胞，需要经验丰富的操作者，且不停地调焦来鉴别；可想而知要把每个红细胞鉴别出来，要耗费多长的时间，需要多强的工作量。



PBMC 样本的差异性大

由于个体的差异（如正常人和病人）和人工分离提取的差异，红细胞和血小板污染的程度差异性非常大，因此我们经常看到分离后的 PBMC 样本千差万别（见下图），这也大大增加了在同一条件下，简单快速准确计数 PBMC 的难度和检测 PBMC 活性的难度。



二、 PBMC 精确计数或活力分析的重要性

PBMC（外周血单个核细胞）是免疫学功能研究中最常用的细胞模型，如细胞增殖、细胞毒性、细胞因子分泌等。

如在癌症免疫治疗领域中，需从病人全血中分离出 PBMC，分离的 PBMC 进一步扩增或进行不同的功能检测。激活扩增后的细胞再回输到病人体内进行细胞治疗。在整个流程中，从分离到检测，到培养，到回输等过程，细胞浓度和细胞活力都是必须监测的参数。

密度梯度分离液是从外周血、骨髓，及脐血分离单个核细胞最常用的方法。但是不可避免的，分离后的细胞中，会有残存的红细胞及血小板混合在单个核细胞中。残存的红细胞和血小板的多少，与

个体的差异，以及分离的效果相关；但是不管分离的技术多高超，经验多丰富，都不可避免会存在红细胞和血小板的污染。

PBMC 实验的特征

1. 随着时间的延长，细胞质量下降；
2. 临床试验相关的样品量很大；
3. 细胞样品不纯，分离之路依赖病人样本和操作者

如何精确、快速、简便计数 PBMC 以及精确分析 PBMC 活力，是免疫学研究以及免疫治疗领域至关重要的步骤。

1. 使用血球计数板在显微镜下进行手工计数 PBMC，即耗时耗人力，更不能达到精确计数的目的。
2. 通过明场的常规细胞计数仪同样不能区分红细胞，不能达到精确计数 PBMC 和精确活力分析的目的。
3. 迫切需要快速、简便、精确计数 PBMC 的工具替代人工计数。Nexcelom 公司走在了市场领先的位置，在常规明场的细胞计数仪基础上，开发出了双荧光细胞活力分析仪，可通过 AOPI 染料进行 PBMC 的快速精确计数和活力分析。

参考文献

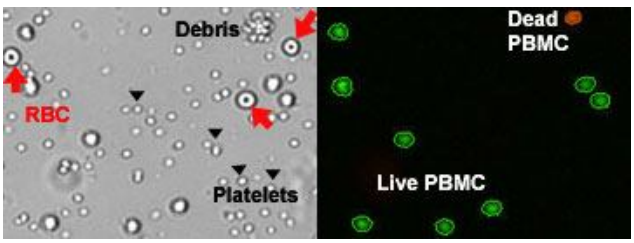
1. Preliminary Report: Evaluation of Storage Conditions and Cryococktails during Peripheral Blood Mononuclear Cell Cryopreservation", L.M. Cosentino, W. Corwin, J.M Baust, N.Diaz-Mayoral, H.Cooley, W. Shao, R. Van Buskirk, and J.G Baust, Cell Preservation Technology, Volume 5 Number 4, 2007
2. "Viability and Functional Activity of Cryopreserved Mononuclear Cells", A. Weinberg, L. Zhang, D. Brown, A. Brice, B.Polsky, M. S. Hirsch, S. Owens, and K. Lamb Clincal and Diagnostic Laboratory Immunology, July, 2000, P714-716
3. "Cell loss and recovery in umbilical cord blood processing: a comparison of postthaw and postwash samples", V. Laroche, D. H. McKenna, G. Moroff, T. Schierman, D. Kadidlo, and J. McCullough, Transfusion, Vol., 45, Dec. 2005.
4. "Viability and Recovery of Peripheral Blood Mononuclear Cells Cryopreserved for up to 12 Years in a Multicenter Study", C. A. Kleeberger, R. H. Lyles, J. B. Margolick, C. R. Rinaldo, J. P. Phair, and J. V. Giorgi, Clinccal and Diagnostic laboratory Immunology, Vol. 6, No. 1, Jan. 1999.

三、 如何精确计数 PBMC 和活力分析检测

通过 AOPI 染料进行双荧光计数和活力分析，是可以排除红细胞、血小板、细胞碎片等污染的精确计数方法。

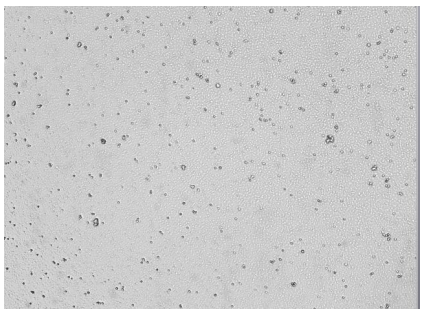
AO（吖啶橙）和 PI（碘化丙啶）是可对 DNA 染色的细胞核染色试剂。其中 AO 可以通过完整的细胞膜，嵌入所有细胞（活细胞和死细胞）的细胞核，呈现绿色荧光；PI 只能通过不完整的细胞膜，即死细胞的细胞膜，嵌入所有死细胞的细胞核，呈现红色荧光。

活死单个核细胞可呈现荧光信号。而成熟的红细胞及血小板，因为没有细胞核，不能被 AO/PI 染色，因此可以完全被排除在外不被计数。

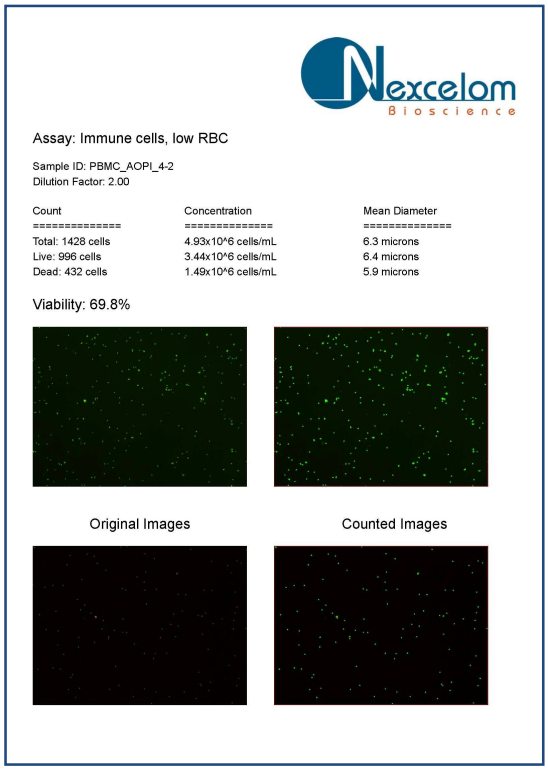


通过 AOPI 染料进行双荧光计数和活力分析，可以精确计数分离后的 PBMC 以及活力分析，亦可进行全血中的 PBMC。

分离液后样本PBMC计数&活性分析举例

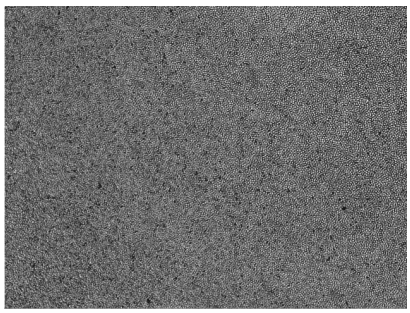


明场图像显示 RBC/血小板污染

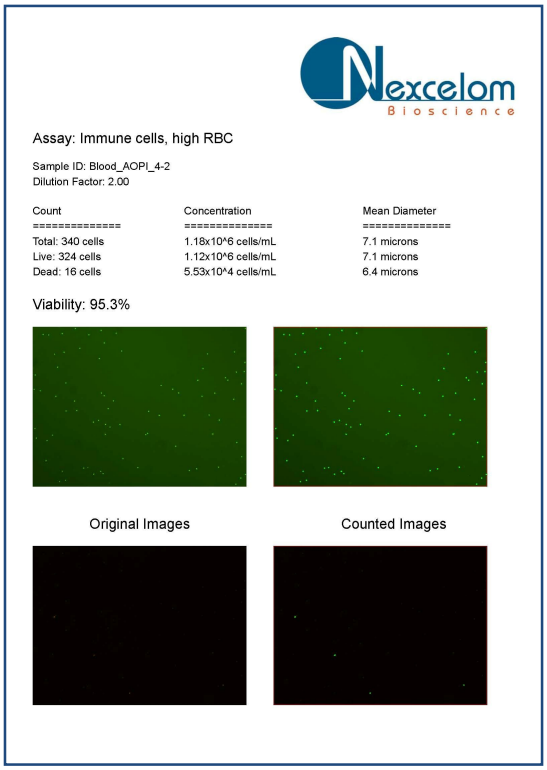


双荧光活力分析仪结果报告输出

全血样本PBMC计数细胞及活性分析举例



明场图像根本无法计数



双荧光活力分析仪结果报告输出

四、 Cellometer 细胞计数和活力分析仪器型号选择

1. 双荧光计数和活力分析是 PBMC 计数的最佳选择方法。
   三款仪器型号可选：AUTO2000/K2/ VISION CBA，可精确、快速、简便进行 PBMC 计数和活力分析。

AUTO2000 双荧光细胞活力分析仪

触屏操控

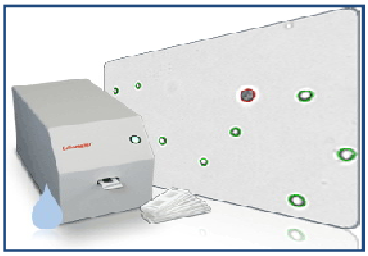
K2 双荧光细胞分析仪

电脑操控

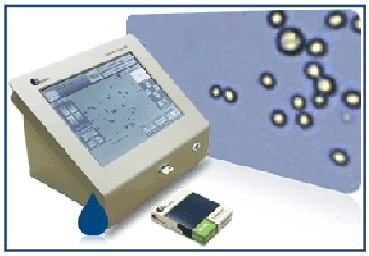
VISION CBA 细胞功能分析系统

电脑操控，高配置，可分析凋亡&细胞周期等

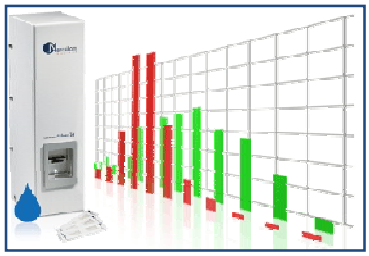
2. 明场的自动细胞计数仪进行 PBMC 计数，可通过台盼蓝排斥法进行 PBMC 的活力检测。
虽然也能达到自动、快速的目的，但是仍无法有效的排除红细胞的干扰，达到精确计数的目的。
台盼蓝排斥法检测细胞死活，是通过台盼蓝这个细胞活性染料，其不能透过活细胞正常完整的细胞膜，故活细胞不着色，但死亡细胞的细胞膜通透性增加，可使染料通过细胞膜进入细胞内，使死细胞着色呈蓝色。是最常用的检测细胞活率的方法。
但是台盼蓝排斥法并不能精确计数活死细胞，原因是细胞膜通透性不一样，进入细胞内的染料差异也很大，所以经常会出现很难判定是死细胞还是活细胞。
如果对 PBMC 计数的准确性要求不是很高，但是需要快速、自动计数，而且对实验的一致性和重复性的要求高，可选择以下三款计数仪：AUTO1000/MINI/AUTO T4。



MINI 自动细胞计数仪
电脑操控，小巧美观，性价比高



AUTO1000 一体式细胞计数仪
一体化设计，触屏操控



AUTO T4 自动细胞计数仪
经典款，符合 GLP/GMP

参考文献

1. Chan, L.L., Wilkinson, A.R., Paradis, B.D. and Lai, N. (2012b) Rapid Image-based Cytometry for Comparison of Fluorescent Viability Staining Methods. Journal of Fluorescence 22, 1301-1311.
2. Almeida, C.-A.M., Roberts, S.G., Laird, R., McKinnon, E., Ahmed, I., Pfafferott, K., Turley, J., Keane, N.M., Lucas, A., Rushton, B., Chopra, A., Mallal, S. and John, M. (2009) Automation of the ELISpot assay for high-throughput detection of antigen-specific T-cell responses. Journal of Immunological Methods 344, 1-5.
3. Constantino, B.T. and Cogionis, B. (2000) Nucleated RBCs - Significance in the Peripheral Blood Film. Laboratory Medicine 31, 223-229.
4. Laroche, V., McKenna, D.H., Moroff, G., Schierman, T., Kadidlo, D. and McCullough, J. (2005) Cell loss and recovery in umbilical cord blood processing: a comparison of postthaw and postwash samples. Transfusion 45, 1909-1916.
5. Sigfusson, A. and Souhami, R. (1984) The Effects of Erythrocyte Contamination on Pokeweed Mitogen Induced Immunoglobulin-Synthesis in Man. Journal of Immunological Methods 72, 167-170.
6. Szabo, S.E., Monroe, S.L., Fiorino, S., Bitzan, J. and Loper, K. (2004) Evaluation of an Automated Instrument for Viability and Concentration Measurements of Cryopreserved Hematopoietic Cells. Laboratory Hematology 10, 109-111.

五、使用仪器发表文章

Author	Date	Title	Journal	Cell Type	Cellometer / Applications
Mahato, Ram I	November 2013	Synthesis and Characterization of an Anti-Apoptotic Immunosuppressive Compound for Improving the Outcome of Islet Transplantation	Bioconjugate Chemistry	PBMC	Cellometer Auto T4... Cell Counting
Banerjee, Subhadra	October 2013	Expression of the B-Cell Receptor Component CD79a on Immature Myeloid Cells Contributes to Their Tumor Promoting Effects	PLOS ONE	PBMC	Cellometer (Not Specified)... Cell Concentration
Bogoslovsky,	September	Cryopreservation and Enumeration of	Blood	PBMC	Cellometer Auto

Tanya	2013	Human Endothelial Progenitor and Endothelial Cells for Clinical Trials	Disorders and Transfusion		T4... Trypan Blue Exclusion
Singh, Harjeet	May 2013	Manufacture of Clincal-Grade CD-19-Specific T Cells Stably Expressing Chimeric Antigen Receptor Using Sleeping Beauty System and Artifical Antigen Presenting Cells	PLoS one	PBMC, Daudi, NALM-6, EL-4	Cellometer Unspecfied... Trypan blue viability
Shankar Pandey, Gouri	January 2013	Detection of Intracellular Factor VIII Protein in Peripheral Blood Mononuclear Cells by Flow Cytometry	BioMed Research International	PBMCs	Cellometer Unspecfied... Trypan blue viability
Filbert, Helene	October 2012	Serum-free freezing media support high cell quality and excellent ELISPOT assay performance across a wide variety of different assay protocols	Cancer Immunology and Immunotherapy	PBMC	Nexcelom Cellometer... Trypan blue viability
Duran, M.C.	July 2012	Enhanced protocol for CD14+ cell enrichment from equine peripheral blood via anti-human CD14 mAb and automated magnetic activated cell sorting	Equine Veterinary Journal	PBMCs	Cellometer Auto T4... Trypan blue viability
O'Connor, Colleen M.	February 2012	Adoptive T-cell therapy improves treatment of canine non-Hodgkin lymphoma post chemotherapy	Scientific Reports	T-cells and PBMC	Cellometer Auto T4... Trypan blue viability
Clark, Eva H.	January 2012	Plasmodium falciparum Malaria in the Peruvian Amazon, a Region of low Transmission, Is Associated with Immunologic Memory	Infection and Immunity	PBMC	Cellometer Auto T4... Trypan blue viability
Sethu, Palaniappan	December 2011	Clinical application of microfluidic leukocyte enrichment protocol in mild phenotype sickle cell disease (SCD)	Biomedical Microdevices	Leukocyte-enriched sample, PBMC	Cellometer (Not Specified)... Cell Number
Uhl, Elizabeth	August 2011	Identification of Altered MicroRNA Expression in Canine Lymphoid Cell Lines and Cases of B- and T-Cell Lymphomas	Genes, Chromosomes, & Cancer	GL-1; CL-1, LN, PBMC	Cellometer Auto T4... Other
Lee, Moo-Seung	June 2011	Shiga toxins induce autophagy leading to differential signalling pathways in toxin-sensitive and toxin resistant human cells	Cellular Microbiology	Primary PBMCs, THP-1	Cellometer Automated Cell counter... Other
Zierold, Claudia	May 2011	Developing mechanistic insights into cardiovascular cell therapy: Cardiovascular Cell Therapy Research Network Biorepository Core Laboratory rationale	American Heart Journal	Bone Marrow, PBMC	Cellometer Auto T4... Trypan blue viability
Pathak, Shresh	February 2011	IL-1B; Is Overexpressed and Aberrantly Regulated in Corticosteroid Nonresponders with Autoimmune Inner Ear Disease	The Journal of Immunology	PBMC	Cellometer Auto T4... Trypan blue viability
Wullner, Danika	June 2010	Considerations for optimization and validation of an in vitro PBMC derived T cell assay for immunogenicity prediction of biotherapeutics	Clinical Immunology	PBMC, Dendritic, CD4	Cellometer Auto T4... Trypan blue viability
Bruchova, Hana	March 2009	Erythropoiesis in polycythemia vera is hyper-proliferative and has accelerated maturation	Blood Cells, Molecules, and Diseases	PBMC	Cellometer Auto T4... Trypan blue viability

Meyers, John A.	February 2009	Chronic Lymphocytic Leukemia and B and T Cells Differ in their Response to Cyclic Nucleotide Phosphodiesterase Inhibitors	The Journal of Immunology	CLL cells, PBMC	Cellometer Auto T4/M10... Normalizati
White, William N.	December 2008	Clinical application of microfluidic leukocyte enrichment protocol in mild phenotype sick cell disease (SCD)	Biomedical Microdevices	PBMC, PNM, Erythrocytes	Cellometer Auto T4... Other
Bruchova, Hana	August 2007	Regulated expression of microRNAs in normal and polycythemia vera erythropoiesis	Experimental Hematology	PBMC, JAK2 V617F	Cellometer Auto T4... Trypan blue viability

详细信息请咨询：

达科为生物技术有限公司

联系人：肖女士

电话：0755-26410151

邮箱：equipment@dakewe.com

网址：www.dakewe.com