浙江大学爱丁堡大学联合学院公共技术平台流式分选服务指南
Guidelines for Using Cell Sorter of ZJU-UoE Core Facility

一、 注意事项 Notice

1. 分选所用的细胞染色方法与分析所用方法基本相同，但需要注意以下几点:The cell staining methods used for sorting are basically the same as those used for analysis, but the following points need to be noted：

   a. 因分选得到的细胞需继续培养，所以在使用 BD Influx 流式细胞分选仪之前，需保证样本无细菌、真菌、病毒和支原体等感染，避免管路污染；Because the cells obtained by sorting need to be continuously cultured, before using the BD Influx, the samples must be free of bacteria, fungi, viruses and mycoplasma to avoid contamination of the pipeline;

   b. 不能使用固定剂固定细胞，因为活细胞经固定剂处理后即死亡；Do not use fixative to fix cells, because fixative can make the living cells die;

   c. 保证上机样品为单细胞悬液；Ensure that the sample is a single cell suspension;

   d. 准备充足的细胞，详见 Table 1；Prepare enough cells, see Table 1 for details;

   e. 上样缓冲液一般建议使用 PBS。若细胞活性较差，可分选上样管中的缓冲液使用含 2% FBS 的 PBS，普通培养基中的酚红可能会干扰分选；若用培养基的话，颜色不能太深，血清浓度不能超过 2%，否则粘性太大影响分选。The loading buffer is generally recommended to use PBS. If the cell viability is poor, the buffer in the sample tube can be sorted using PBS containing 2% FBS. Phenol red in the common medium may interfere with the sorting; if the medium is used, the color should not be too dark and the serum concentration Can't exceed 2%, otherwise the viscosity is too big to affect sort.

   f. 如细胞分选后需再次培养，请准备含血清的收集管，在分选前交管理员。建议在 5ml 离心管中加入 1-2ml 血清及其它必需组分，保证分选完毕时血清浓度大于 5%；If cells need to be cultured again after sorting, please prepare a collection tube containing serum and give it to the administrator before sorting. It is recommended to add 1-2ml serum and other necessary

components to the 5ml centrifuge tube to ensure that the serum concentration is greater than 5% after sorting;

g. 如要分选 GFP 等转染的样品，请提供未经转染的相同细胞为阴性对照；If you want to sort GFP labeled or other fluorochrome stained samples, please provide the same non-transfected cells as a negative control.

h. 如要做多重荧光染色标本的分选，请提供各种单一荧光染色的标本。如要去除死细胞，在不影响后续实验前提下，可以加入 7-AAD、PI 或 DAPI； For sorting of multiple fluorescent stained specimens, please provide a variety of single fluorescent stained specimens.To remove dead cells, add 7-AAD, PI or DAPI.

i. 快速简便的样本处理有利于分选，处理好的样本尽快上机，分选好的细胞尽快下一步实验，如果要分选多个样本，建议一次处理一个，估计可能的上机时间后，再处理第二个样本。Handling samples quickly is conducive to sorting: the processed samples are put on the machine as soon as possible, and the sorted cells are used for your experiments as soon as possible. If you want to sort multiple samples, it is recommended to process one at a time.

2. 数据拷贝：实验结束后及时用格式化的 U 盘或光盘、FTP 拷贝相关实验数据，原始数据只保留 1 个月；禁止未经许可拷贝他人数据。Data copying: After the experiment is over, use formatted USB, CD or FTP to copy the relevant experimental data in time. The original data is only kept for 1 month. It is forbidden to copy the data of others without permission.
3. 使用登记：上机完后用户需进行使用登记，及时清理实验台面，严格规范使用仪器。Use registration: users need to register after using the instrument, clean up the experimental table in time, and strictly follow the rules.
4. 请用户上机前仔细阅读有关使用说明、管理规定及注意事项，自助操作的用户需严格遵守仪器使用规范。用户发现仪器损坏或故障的，或因个人操作不当等导致仪器损坏或故障的，应当及时告知仪器管理员，不得隐瞒。Please read the relevant instructions and guidelines carefully before using the instruments especially for those who operate instruments by themselves. If you find that the

instrument is damaged or broken, please inform the instrument administrator promptly, and do not conceal it.

5.

Table 1 Nozzle Selection with Different Cell Types&Concentrations

Cell Types and Size (Diameter)	Concentrate	Nozzle
Lymphocytes, thymocytes or splenocytes,etc. (8-12μm)	8-12×10^6/ml	70/86μm
Activated lymphocytes, smaller cell lines, cancer cells, etc. (12-20μm)	7-9×10^6/ml	86/100μm
Large adherent cell lines, etc. (>20μm)	5-10×10^6/ml	140μm

二、 常见问题 Frequently Asked Questions

Q1. 上机样品需要溶在何种溶液中？是否可以就直接放在原本培养的培养基中？Sample needs to be dissolved in what kind of solution? Can it be placed directly in the original culture medium?

Ans: 建议不要放培养基中，因为 Phenol Red 可能会影响分选的结果，可先尝试使用含 2% FBS 或 BSA 的 PBS 作为分选缓冲液。如要求更好的细胞存活率，按分选细胞的不同，选择不同的分选缓冲液。It is recommended not to put it in the medium, because Phenol Red may affect the sorting results, you can try to use PBS containing 2% FBS or BSA as the sorting buffer. If better cell survival rate is required, select different sorting buffers according to the samples.

1. 淋巴细胞（HBSS 配方中的阳离子可增进细胞的生存力。如果这些细胞并非易于群集细胞，可以选用没有 EDTA 的缓冲液）。Lymphocytes (the cations in the HBSS formula can improve the viability of cells. If these cells are not easy to cluster, you can choose a buffer without EDTA).

   a. 1 mM EDTA, 25mM Hepes (pH 7.0) and 0.5% - 2% BSA in 1× PBS (without Ca^{2+} and Mg^{2+})

   b. 1× HBSS (without phenol red) with 1% BSA

   c. 0.5%-2% BSA in PBS (without Ca^{2+} and Mg^{2+})

2. 贴壁细胞（以 Trypsin 处理后去准备单细胞悬浮液时，待细胞变圆(切记不可过度作用)，以适量含 5%血清培养液收取细胞，并均匀地打散细胞悬浮液。离心后，用分选缓冲液调整细胞浓度。如果需要的话可以提高 EDTA 的浓度

(至 5mM）以避免细胞重新黏聚。Adherent cells (when using Trypsin to prepare a single cell suspension, wait for the cells to become round (remember not to over-act), collect the cells with an appropriate amount of 5% serum culture medium, and evenly disperse the cell suspension. After centrifugation, adjust the cell concentration with sorting buffer. If necessary, increase the concentration of EDTA (to 5mM) to avoid re-aggregation of cells.

a. 5 mM EDTA, 25mM Hepes (pH 7.0) and 0.5% - 2% BSA in 1× PBS (without Ca^{2+} and Mg^{2+})

b. 0.5-2% BSA in PBS (without Ca^{2+} and Mg^{2+})

3. 含有高比例死细胞的样本（这些配方可减少因死细胞释放出来的 DNA 所造成的细胞黏聚现象）。Samples containing a high proportion of dead cells (The formula can reduce cell adhesion caused by DNA released from dead cells).

5mM $MgCl_2$, 1 mM EDTA, 25mM Hepes (pH7.0), 25-50 μg/ml DNAase I and 0.5% - 2% BSA in 1× PBS (without Ca^{2+} and Mg^{2+})

4. 建议询问有经验者（使用相同细胞进行过分选）的分选缓冲液配方。It is recommended to ask those who used the same kind of cells for sorting and know the buffer formulations for help.

Q2. 如果细胞要再培养，会污染吗？Will the cells be contaminated if they are to be cultured?

Ans: 分选所用的所有溶液都经高温高压灭菌，流式分选仪管路使用 70%乙醇或次氯酸钠定期清洗，仪器所在房间在每次分选前紫外灭菌，若在细胞培养基中加入抗生素，可将污染机率降至很低。All solutions used for sorting are sterilized by high temperature and high pressure. The pipeline of the flow sorter is regularly cleaned with 70% ethanol or sodium hypochlorite. The room where the instrument is located is sterilized by UV before sorting. Antibiotics are added to the cell culture medium, which is helpful to reduce the probability of pollution.

Q3. 如果我想在分选后拿到 1×10^6 的细胞我应该一开始准备多少细胞去做分选？If I want to get 1×10^6 cells after sorting, how many cells should I prepare?

Ans: 假设你要的细胞只占总数的 10%，则你所需准备的细胞数如下公式: 起始细胞数=1×10^6/ (10%× 50%回收率)，所以你需要准备 2×10^7 细胞。高速分选、纯

度模式(purity vs. yield mode)、部分细胞黏附于上样管壁、部分细胞用于样本分析、长时间分选过程中细胞的死亡等等，都会降低回收率。50%回收率是一般参考值。Assuming that the cells you want only account for 10% of the total, the number of cells you need to prepare is as follows: the number of cells = 1×10^6/ (10% ×50% recovery rate), so you need to prepare 2×10^7 cells. Something like sort speed, sort mode(purity vs. yield) and cell viability can reduce the recovery rate,so 50% is a reference value in general.

Q4. 通常做一次细胞分选需要多久时间？How long does it take for a cell sorting usually?

Ans: 分选的过程有三个步骤，分别为设定分选区域、分选及分选后的分析。设定分选区域约需 15-20 分钟。分选的时间决定于细胞数目，虽然机器的分选速度最高可达 70,000 个细胞/秒，但为得到最佳分选结果，我们通常设定分选速度为 5,000-6,000 个细胞/秒。因此，如欲分选 1×10^7 细胞，其所需分选时间约为 40 分钟。分选后约需 15-20 分钟去回测及分析结果。所以，总共约需 1 小时去分选 1×10^7 细胞。There are three steps for sorting: gating, sorting and data analysis. It takes about 15-20 minutes to gate. The time for sorting depends on the number of cells. Although the sorting speed can be up to 70,000 cells/s, we usually set the sorting speed to 5,000-6,000 cells/s to achieve the best sorting effect. Therefore, it takes about 40 minutes to sort. What's more, it takes about 15-20 minutes to sort back and analyze the results after sorting. In total, it takes about an hour to sort 1×10^7 cells.

Q5. 一个样品可以同时分选出几种细胞? How many kinds of cells can be sorted in one sample simultaneously?

Ans: BD Influx 可以从一个样品中最多同时分选出 6 种不同的细胞。For BD Influx, up to 6 different cells can be sorted from a sample at the same time.

Q6. 可以同时使用多少参数进行细胞分选？How many parameters can be used for cell sorting at the same time?

Ans: BD Influx 最多可以根据 21 个参数去定义及分选一群细胞（前侧向散射光 SSC 和 FSC、355nm 激光对应的 2 种荧光通道信号、405nm 激光对应的 6 种荧光通道信号、488 nm 激光对应的 3 种荧光通道信号、561nm 激光对应的 5 种荧

光通道信号和 640nm 激光对应的 3 种荧光通道信号）。When using BD Influx, up to 21 parameters can be used to sort cells(FSC&SSC, 2 colors for 355nm laser, 6 colors for 405nm laser, 3 colors for 488nm laser, 5 colors for 561nm laser and 3 colors for 640nm laser).

Q7. 分选后细胞的纯度有多少？What is the purity of cells after sorting?
Ans: 通常可以达到 95%以上的纯度，如果所分选细胞可以和其他细胞群较好的区分。If the sorted cells can be distinguished from other cell populations easily, it can achieve a purity of more than 95%.

Q8. 如果阳性的细胞占总数的 1%以下，还可以做分选吗？If the positive cells account for less than 1% of the total, can it still be sorted?
Ans: 可以，但是低含量细胞的分选会导致低纯度及低回收率。因此，我们建议使用者事先富集你感兴趣的细胞。细胞富集的方式可以是正选，如用磁珠法富集你感兴趣的细胞；也可以是负选，如利用 nylon wool 去除 B 细胞，或磁珠法去除不要的细胞。Yes, but the sorting of low-content cells may result in low purity and low recovery. Therefore, we recommend that users enrich cells of interest in advance. The method for enriching can be positive selection, for example, using magnetic bead to enrich the cells of interest, or it can also be negative selection, such as using nylon wool to remove B cells, or using magnetic bead to remove unwanted cells.

Q9. 分选时所使用的管子有哪些？What are the tubes used for sorting?
Ans: 上样一般使用 5ml 带盖流式管(BD Falcon tube #352063)，收集容器主要有以下几种：5ml Falcon tubes(#352063) is generally used for sample loading, and collection devices mainly have the following types:
Two-way sorting： 1.5-ml, 5-ml, 15-ml, and 50-ml tubes, and 25-mm round filter paper; **Three-way sorting:** One 50-ml tube and two 5-ml tubes; **Four-way sorting:**1.5-ml and 5-ml tubes；**Six-way sorting:** 5-ml tubes，Plates and slides: 6, 24, 48, 96, and 384-well plates，slides and user-defined collection devices.