Application Note

INTEGRA

Precise and reproducible automated cell seeding with VIAFLO 96 and VIAFLO 384 handheld electronic pipettes

Introduction

Cell culture is a technique in which cells are grown outside a living organism under well-defined, controlled conditions. It is important for basic biological research and a wide range of clinical studies. Mass culture of cells is indispensable for producing viral vaccines, enzymes, synthetic hormones, anticancer agents, recombinant proteins and antibodies, but also for studying the physiology and biochemistry of cells. Nowadays, cell culture is also used in agriculture for producing alternative foods. Preserving cell viability, avoidance of contamination, high experimental reproducibility, and accuracy are vital for manipulations carried out on cells during media changes, transfers, seeding and passaging. All these can be mastered with VIAFLO 96 and VIAFLO 384 handheld electronic pipettes.

Key benefits:

- This protocol is automated for maximum reproducibility. Reducing the hands-on time for laboratory personnel can help to diminish the risk of contamination.
- The optimized z-height and mixing step set-up eliminates user variability. This is crucial to maximize viability during cell culture.
- Pipetting is carried out at an accurate and consistent speed to minimize the shearing forces that cells are subjected to.
- The VIAFLO 96 and VIAFLO 384 combine all the benefits of VIAFLO pipettes with the ability to pipette 24, 96 or 384 channels simultaneously, for unmatched throughput and precision.

Overview: mouse embryonic stem cell culturing

Experimental set-up

In this protocol, ES-E14TG2 mouse embryonic stem cells (Sigma) are washed, harvested and transferred to a new plate with fresh media, using a VIAFLO 384 handheld electronic pipette with a three position stage (with positions A, A/B and B, **Figure 1**). For the aspiration steps, a VACUBOY vacuum hand operator is used in combination with a VACUSAFE aspiration system.

A 96 channel pipetting head (10-300 µl) is used together with 300 µl Sterile, Filter GripTips. Customized VIALINK programs are provided to perform cell washing, harvesting, seeding and splitting with a VIAFLO 384.

Note: The VIALINK programs provided can be easily adapted to culture other cell types by adding or removing steps from the protocols supplied.

VIAFLO 384

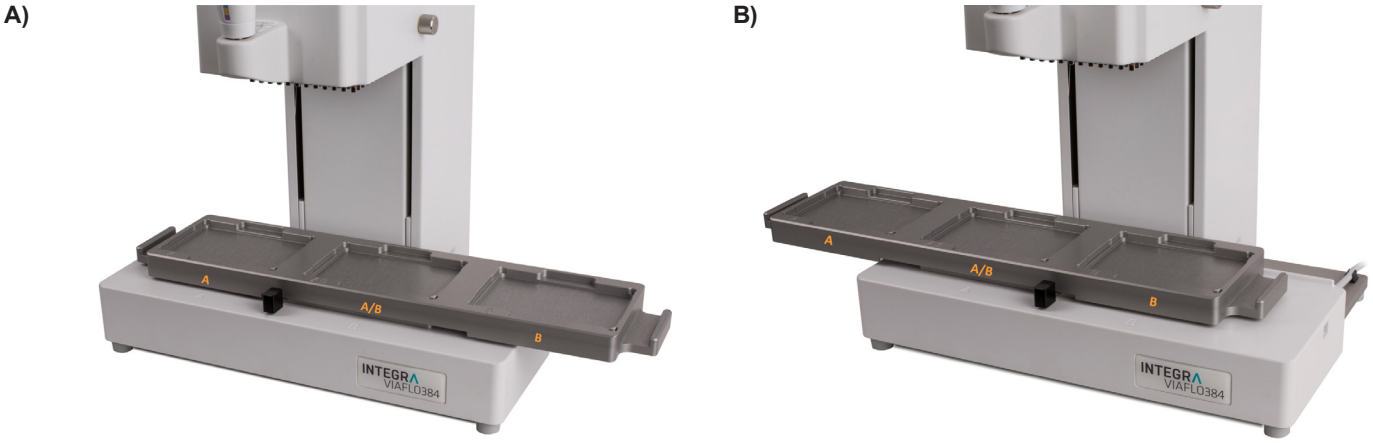

Figure 1: Three position stage of VIAFLO 96 and VIAFLO 384. a) position 1: position A and A/B can be reached, b) position 2: position A/B and B are reachable.

Overview of the process

Step 1: Washing the cells
Step 2: Addition of detaching reagent
Step 3: Incubation and new media addition
Step 4: Splitting the cells
Step 5: Triplicating the cells

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1. Washing the cells

STEP: Washing the cells with 1x PBS.

HOW TO: Load a full box of 300 µl Sterile, Filter GripTips on the VIAFLO 384. Slide the three position stage to the left so it is in position 2 (A/B-B). Place a 300 ml automation friendly reagent reservoir with 1x PBS in position AB.

Aspirate the cell culture media (100 µl) from the 96 well culture plate using a VACUBOY in combination with a VACUSAFE (**Figure 1**).

After aspirating the cell culture media, place the plate on position B of the VIAFLO 384 and launch the custom VIALINK program 'PBS_WASH'. The pipette aspirates 80 µl 1x PBS from the automation friendly reservoir and dispenses it into the 96 well culture plate.

Figure 2: Aspirating the cell culture media using a VACUBOY vacuum hand operator.

2. Addition of detaching reagent	**STEP:** Addition of accutase solution to detach the cells	**HOW TO:** Load a full box of 300 µl Sterile, Filter GripTips on the VIAFLO 384. Place the 300 ml automation friendly reagent reservoir with the accutase solution in position AB, with the stage in position 2. Aspirate the 1x PBS from the culture plate using the VACUBOY with the VACUSAFE. After aspirating the 1x PBS, place the 96 well culture plate on position B of the VIAFLO 384. Select and run the custom VIALINK program 'ACCUTASE_ADD'. The pipette aspirates 40 µl accutase solution from the automation friendly reagent reservoir and dispenses it into the 96 well cell culture plate.
3. Incubation and new media addition	**STEP:** Incubation of the cell culture plate and preparation of a new plate with fresh cell culture medium	**HOW TO:** Incubate the plate for 15 min at 37 °C with 8 % CO_2 in the incubator to allow the accutase to detach the cells from the plate surface. During the incubation of the cell culture plate, a new 96 well culture plate with fresh cell culture medium can be prepared. Load a full box of Sterile, Filter 300 µl GripTips on the VIAFLO 384. Place the 300 ml automation friendly reagent reservoir with the fresh cell culture media on position AB, with the stage in position 2. Take a gelatin-coated (either self-made or commercial) 96 well plate and remove the gelatin with the VACUBOY and the VACUSAFE. Place the new 96 well plate in position B of the VIAFLO 384. Launch the custom VIALINK program 'MEDIA_ADD'. The pipette aspirates 100 µl of fresh cell culture medium and dispenses it into the 96 well plate. After a 15 minute incubation, the 96 well culture plate should be examined under the microscope to check that cell detachment is complete. If the cell detachment is complete, there are two options of how to continue. In step four the user can passage the cells – splitting the cells in 1:3 ratio to a new plate. Or, in step five, the user can split the cells in 1:3 ratio, triplicating it into three new plates. **Tip:** • The incubation parameters should be changed depending on the cell type and the detaching reagent.

4. Splitting the cells	**STEP:** Passaging the cells in 1:3 ratio	**HOW TO:** Load a full box of Sterile, Filter 300 µl GripTips on the VIAFLO 384. Place the 300 ml automation friendly reagent reservoir with the fresh cell culture media on position AB, with the stage in position 2. Place the 96 well plate to be split on position B. Place a new 96 well plate on position A. Select and run the custom VIALINK program 'MIX_PLATE'. The VIAFLO 384 aspirates 80 µl fresh cell culture medium and dispenses it into the 96 well plate. It will be followed by a 30-cycle mixing step. When the mixing is finished, the VIAFLO 384 aspirates 40 µl of the cell suspension. The instrument will prompt the user to move the three position stage to position 1, so that position A can be reached. The pipette dispenses 40 µl of cell suspension to the new 96 well culture plate. **Tips:** • In the mixing step, 150 µl of cell suspension is mixed, which is more than the volume in the wells. This creates bubbles, which helps to homogenize the cell suspension. This is a very efficient solution for ES-E14TG2 mouse embryonic stem cells but can be changed for some other cell lines. • The z-height limits are defined in the program to ensure optimal tip immersion depth, preventing the pipette tips from touching the bottom of the plate.
5. Triplicating the cells	**STEP:** Splitting the cells in 1:3 ratio onto three 96 well plates	**HOW TO:** Load a full box of Sterile, Filter 300 µl GripTips on the VIAFLO 384. Place the 300 ml automation friendly reagent reservoir with fresh cell culture medium on position AB, with the stage in position 2. Place the plate to split in position B. Place the first new 96 well plate on position A. Keep the other two new 96 well plates close to the instrument, they will be placed in position A during the program. Launch the custom VIALINK program 'MIX_PLATE_TRI'. 95 µl of fresh cell culture medium will be aspirated and then dispensed into the 96 well plate. The cell culture medium and the cells will be mixed for 30 cycles. When this step is finished, the pipette aspirates 120 µl of cell suspension and alerts the user to change the three stage position, sliding it into position 1. The VIAFLO 384 dispenses 40 µl of cell suspension to a new 96 well plate, then prompts the user to change plate. This step will be repeated two times, resulting in the cells being split into three new plates. **Tip:** • In this example, the ES-E14TG2 mouse embryonic stem cells were split in three. This can easily be set up differently for other cell lines, by changing the volumes of aspiration and dispensing. Experimental results (Friedrich Miescher Institute for Biomedical Research) showed consistency between replicates of mouse embryonic cell samples with the same confluency. The mixing step was very efficient, allowing single cells to be seen under the microscope (**Figure 2c**).

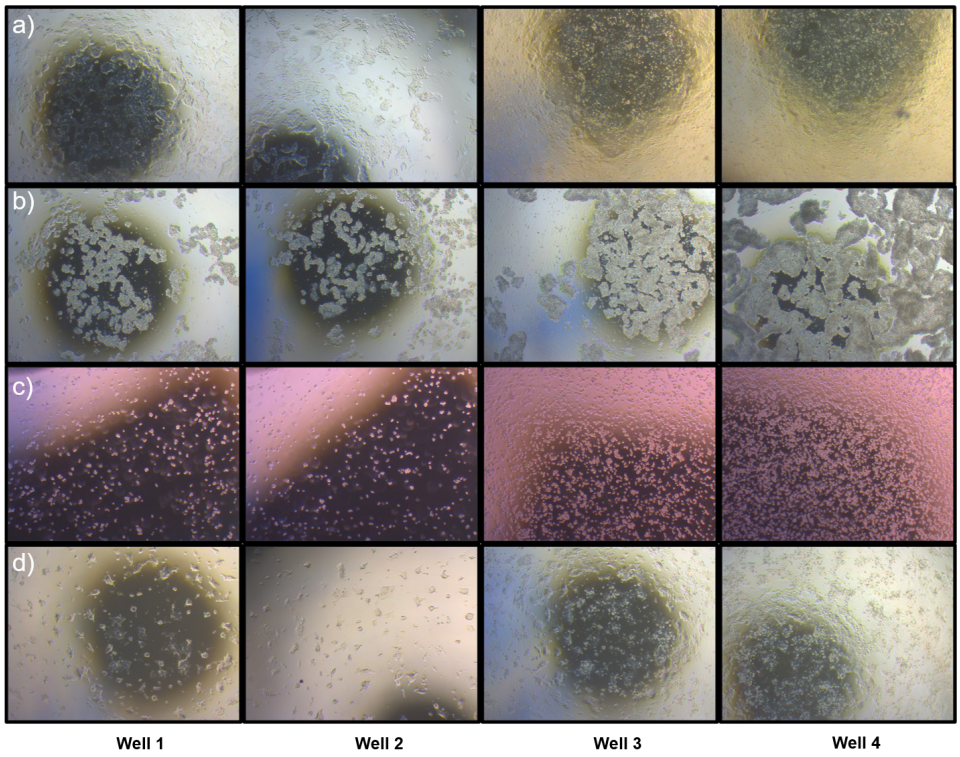

Figure 3: Microscopy picture showing the different stages of the splitting protocol for four different wells. Pictures of mouse embryonic stem cells were taken a) after washing the cells with PBS, b) after the incubation with accutase, c) after splitting the cells, and d) 24 hours after splitting the cells. Wells 1 and 2 showed lower confluence, while wells 3 and 4 had higher confluence. An Axiovert 40 CFL (Zeiss) microscope was used with 2.5x magnification. (Photos courtesy of Julie Cramard, Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland.)

Remark

Partial plates: Programs can be easily adapted to a different number of samples. The VIAFLO 384 is able to work with any number of tips loaded, giving you the benefit of simultaneous and accurate dispensing of a smaller number of samples.

Conclusion

- The VIAFLO 384 offers a simple, accurate, reproducible, and fast pipetting solution for cell washing, transfer and seeding.
- Prolonged manual pipetting tasks can lead to repetitive strain injury. This can be avoided by automating the cell culture steps with the VIAFLO 384, maximizing hands-free time.
- With the optimized electronic mixing and perfect pipetting height control, homogenous cell suspensions are achieved, while minimizing the chance of cell shearing during passaging and splitting.
- The VIAFLO 384 with a 96 channel pipetting head was used in this example, but the VIFALO 96 and VIAFLO 384 base units have easily interchangeable heads to allow 24, 96 and 384 channel pipetting, providing the right tool to match your throughput. The MINI 96 offers another option for this application, but in a much smaller and simpler package.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA	6001/6031	VIAFLO 96/384 handheld electronic pipette	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA	6230	Three position stage for 96 and 384 well plates	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA	6103	96 channel pipetting head 10 - 300 µl	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA	155 500	VACUBOY Vacuum Hand Operator	https://www.integra-biosciences.com/global/en/aspiration-systems/vacuboy
INTEGRA	158310	VACUSAFE Safe Aspiration System	https://www.integra-biosciences.com/global/en/aspiration-systems/vacusafe
INTEGRA	6328	300 ml automation friendly reagent reservoir	https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs
INTEGRA	6435	300 µl Sterile, Filter GripTips	https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide
ECACC	ES-E14TG2a	Mouse embryonic stem cells	https://www.phe-culturecollections.org.uk/products/celllines/generalcell/detail.jsp?refId=08021401&collection=ecacc_gc
Sigma/Merck	D8537	Dulbecco's Phophate Buffered Saline	https://www.sigmaaldrich.com/catalog/product/sigma/d8537?lang=en&region=GB&cm_sp=Insite-_-caSrpResults_srpRecs_srpModel_d8537-1l-_-srpRecs3-1
Sigma/Merck	A6964	Accutase® solution	https://www.sigmaaldrich.com/catalog/product/sigma/a6964?lang=en&region=GB&cm_sp=Insite-_-caSrpResults_srpRecs_srpModel_a6964-_-srpRecs3-1
Sigma/Merck	G5154	Glasgow Minimum Essential Medium	https://www.sigmaaldrich.com/catalog/product/sigma/g5154?lang=en&region=GB&cm_sp=Insite-_-caSrpResults_srpRecs_srpModel_g5154-_-srpRecs3-1
Sigma/Merck	G2500	Gelatin from porcine skin	https://www.sigmaaldrich.com/catalog/product/sigma/g2500?lang=en&region=GB&cm_sp=Insite-_-caSrpResults_srpRecs_srpModel_g2500-_-srpRecs3-1
Sigma/Merck	Z707902-108EA	TPP® tissue culture plate	https://www.sigmaaldrich.com/catalog/product/sigma/z707902?lang=en&region=GB&cm_sp=Insite-_-caSrpResults_srpRecs_srpModel_z707902-_-srpRecs3-1

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