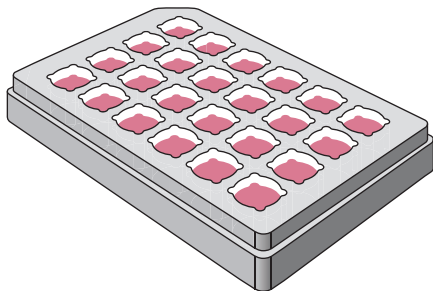
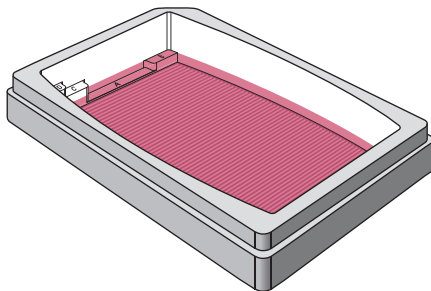


# [U] XDemics

## Expansify™ Quick Start Guide



Expansify™  
24-well plate



Expansify™  
1-well plate

# BEFORE YOU START

Review the following items before using Expansify™ plates. This checklist is intended to support proper setup, consistent handling, and successful use of the plate.

## 1. Review Instructions Before Use

- Watch the XDemics instructional video series prior to handling the plate.
- Read the Expansify™ Quick Start Guide fully before starting.
- Review any application-specific protocol or supplemental guidance provided for your cell type or workflow. These resources are available in the Resources section on the XDemics website and may be used as starting points for experimental design.

## 2. Confirm Required Materials

Before beginning, confirm that the following materials are available:

- Swinging-bucket centrifuge with an adapter compatible with deep-well plates.
- Expansify™ plate or deep-well plate for proper centrifuge counterbalance.
- Aspirating polystyrene Pasteur pipette, or an aspirating pipette fitted with a non-filter 200 µL tip, for accurate liquid manipulation and media removal. Additional handling details are provided on page 6.

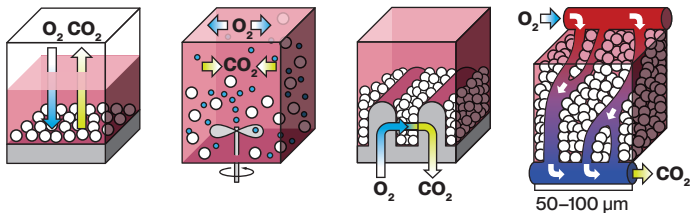
## 3. Recommended Materials

The following materials are recommended to improve ease of use, handling consistency, and culture optimization:

- Wide-bore P1000 tips to reduce cell shear during handling, especially at higher culture densities.
- Glucose and/or lactate meter for extended culture monitoring or optimization.
- ECM coating for adherent cell workflows requiring vessel attachment. For adherent cell workflows using ECM coating, prepare the coated plate prior to seeding and follow the recommended coating procedure. Refer to the coating protocol available in the Resources section on the XDemics website.

# THE OXYGEN DELIVERY BOTTLENECK

Conventional culture systems rely on gas exchange at the liquid-air interface.

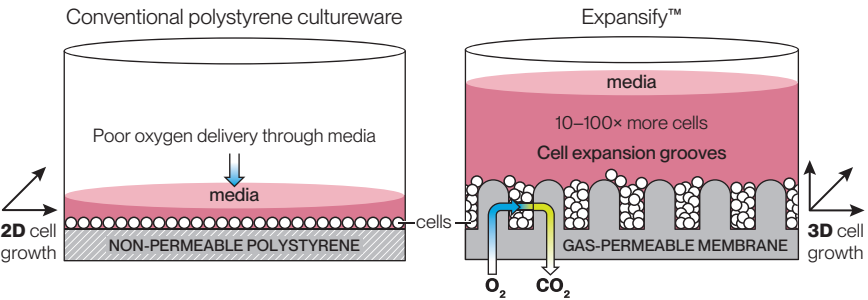


|                                       | Petri dishes, T-flasks | Stirred tank bioreactors | Expansify™ | Tissue |
|---------------------------------------|------------------------|--------------------------|------------|--------|
| Mammalian (million cells / mL)        | ~1                     | ~10                      | >100       | ~460   |
| Oxygen Transfer Rate $k_L a$ (1 / hr) | 0.8                    | 8                        | >100       | ~118   |

$k_L a$  for oxygen is the volumetric mass transfer coefficient, representing the efficiency with which oxygen transfers from gas to liquid.

## THE EXPANSIFY™ SOLUTION

Expansify™ enables tissue-level gas exchange throughout the culture microenvironment.

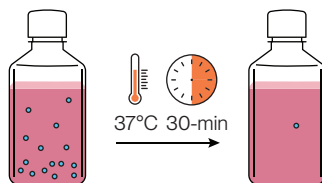


# MEDIA PREPARATION & CELL SEEDING

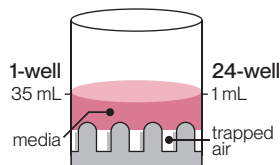
## 1 Pre-warm complete media to 37°C for at least 30 minutes.

Cold media contains more dissolved gas, which may nucleate on the membrane surface.

*(Optional) Degas warm media by vacuum filtration for at least 1 minute to facilitate dissolved gas removal.*



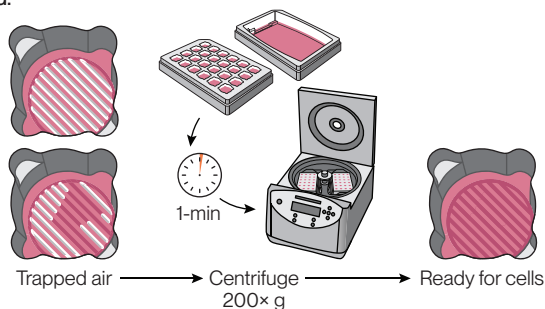
## 2 Add media to the minimum fill volume indicated here:



## 3 Centrifuge at 200× g for at least 1 minute to uniformly remove trapped air from the entire plate. This step cannot be skipped.

### CAUTION

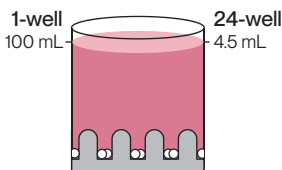
- Do not exceed 500× g.
- Maximum centrifugation volume:  
**24-well:** 3 mL per well.  
**1-well:** 75 mL total volume.
- Properly counterbalance with Expansify™ plate or deep-well plate.



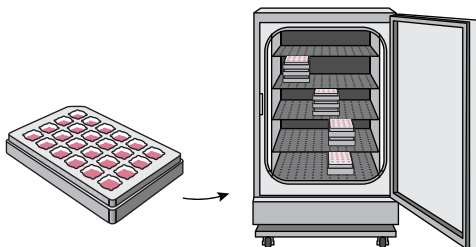
## 4 Prepare a single-cell suspension at your desired seeding density, then add the appropriate volume of cell suspension to each well(s).



## 5 Following cell addition, adjust the culture volume as desired, up to the max fill volume indicated here:



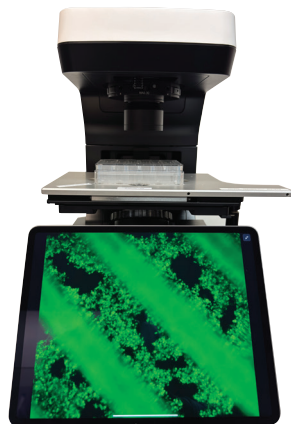
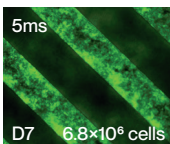
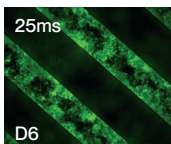
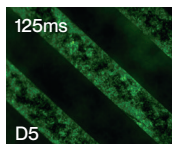
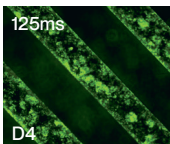
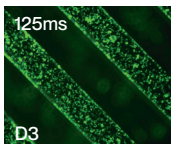
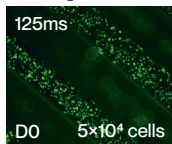
## 6 To ensure homogenous cell distribution, gently mix the cell suspension before returning the plate to the incubator. Cells will uniformly settle into niches over 2–4 hrs.



## IMAGING & MONITORING

Adherent HeLa-GFP cells: 100-fold expansion in Expansify™ 24-well plate.

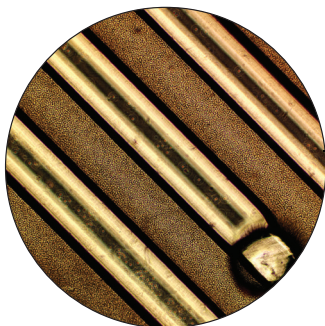
4× magnification



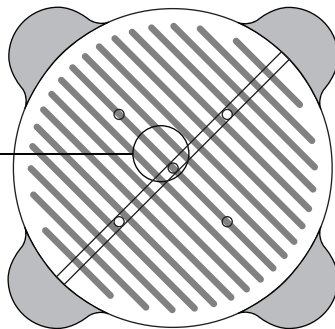
Discover Echo REVOLVE™  
(FITC Channel)

## LOCALIZATION MARKERS (24-WELL ONLY)

Five built-in reference markers enable reproducible positioning and longitudinal imaging of the same regions over time.



K-562 cells at  $2 \times 10^6$  cells/well  
4× magnification

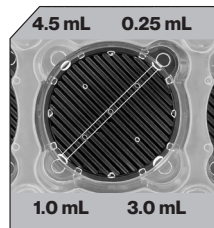


## MEDIA EXCHANGE: EXPANSIFY™ 24-WELL

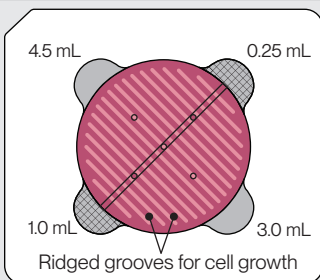
SureShelves™ are precision-engineered shelf graduations integrated within each Expansify™ plate, enabling:

- secure positioning of serological and Pasteur pipettes during media exchange;
- integrated volume graduations for accurate media addition and aspiration;
- consistent culture handling with minimal disruption to cells.

**IMPORTANT:** For optimal volume accuracy, use a fine-tip glass or single-use polystyrene Pasteur pipette. Alternative aspiration devices should be fitted with a fine tip (e.g., a non-filter 200  $\mu$ L micropipette tip) to ensure precise fluid control and minimize disturbance to the cell culture.



SureShelves™



Use the **0.25 mL** and **1.0 mL** SureShelves™ for media addition.

All SureShelves™ can be used for media aspiration.

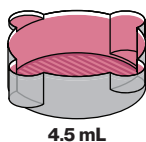
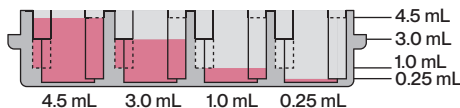
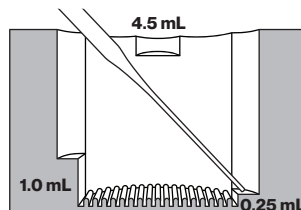
**All Expansify™ plates must remain flat on the work surface to accurately use SureShelves™.**

### Aspiration:

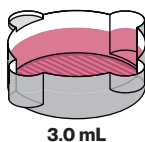
Pasteur pipette must be fully seated at the back of the SureShelves™.

**Aspiration:** use any SureShelves™ to aspirate media, selecting the appropriate shelf based on the desired residual volume.

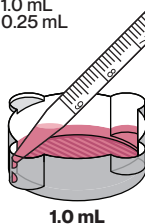
**Addition:** add media at approximately 0.5 mL/s using the 0.25 mL or 1.0 mL SureShelves™, which are oriented perpendicular to the cell expansion grooves to support gentle, controlled media exchange with minimal culture disruption. Use the 4.5 mL SureShelves™ as the maximum media fill indicator.



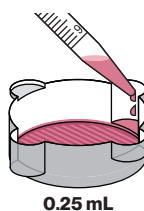
4.5 mL



3.0 mL



1.0 mL



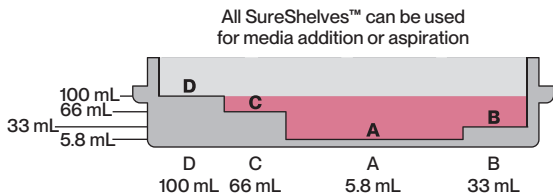
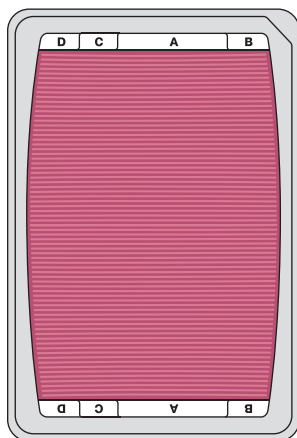
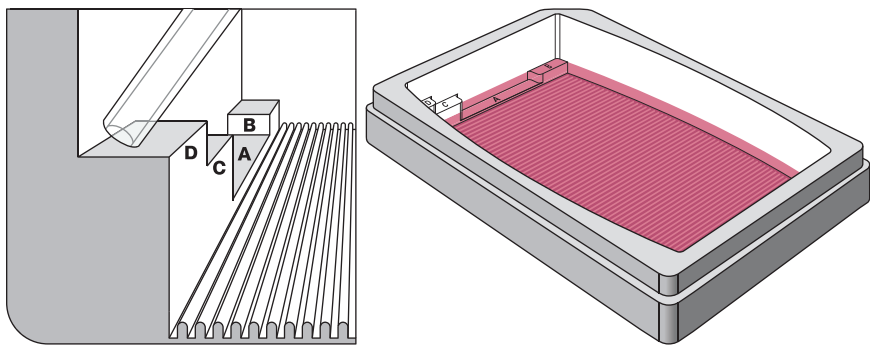
0.25 mL

## MEDIA EXCHANGE: EXPANSIFY™ 1-WELL

SureShelves™ are integrated into the side of the Expansify™ 1-well plate and are labeled A, B, C, and D to facilitate consistent media addition, aspiration, and volume control during culture maintenance.

**Aspiration:** use any SureShelves™ to aspirate media, selecting the appropriate shelf based on the desired residual volume.

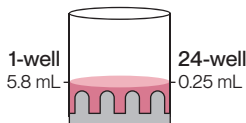
**Addition:** add media at approximately 2 mL/s using any of the SureShelves™. During media exchange, add media from one side and aspirate from the opposite corner to promote fluid flow across the culture surface. Use the 100 mL SureShelves™ as the maximum media fill indicator.



*Volumes may differ slightly due to meniscus formed in the plate*

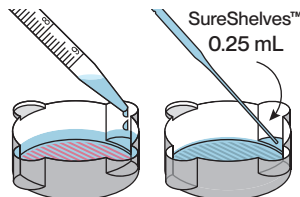
# CELL HARVEST: ADHERENT CELLS

**1 Aspirate** media at the lowest SureShelves™ indicated below.



**2 24-well:** wash the culture surface. Add PBS or preferred wash buffer using the lowest SureShelves™.

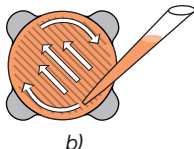
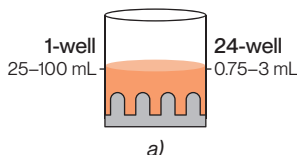
**1-well:** add wash buffer from one side of the plate to promote flow across the culture surface. Aspirate wash buffer using the lowest SureShelves™ from the opposite side of the plate. 1-well plate may be slightly tilted towards the SureShelves™ to remove nearly all buffer.



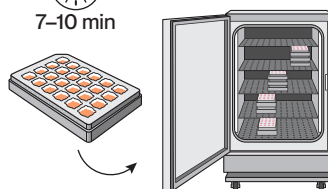
**3 Add** dissociation reagent to each well using the recommended starting volume for the Expansify™ format below.

**Gently mix** to fluidize cells with reagent:

- 24-well: triturate using a P1000 wide-bore tip.
- 1-well: gently mix using a serological pipette.



**4 Incubate** for 7-10 minutes under standard culture conditions.



**5 a)** Remove the plate and gently triturate cell clusters.

*i) At this stage only, the plate may be tilted toward you to facilitate gentle mixing and complete recovery of cells from the well.*

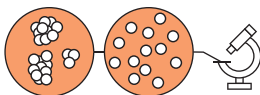
**b)** Check for **dissociation** under the microscope.

*Cell clusters should begin to separate with minimal handling.*

*Avoid excessive pipetting, which may shear cells and reduce viability.*

*Begin the first incubation with a lower dissociation reagent volume (e.g. 0.75 mL), then increase volume only if needed based on observed cell density and remaining cluster burden (e.g. 2 mL).*

*For subsequent incubation + gentle mixing cycles, reagent may be added stepwise up to 3 mL/well for 24-well plates or up to 100 mL for 1-well plates to achieve a uniform single-cell suspension.*



**6 Gently mix** the well contents and proceed immediately with cell counting.

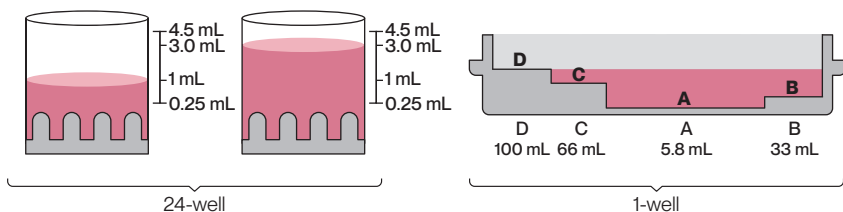
**7 Collect** the cell suspension for downstream processing, analysis, or seeding according to your application-specific workflow.



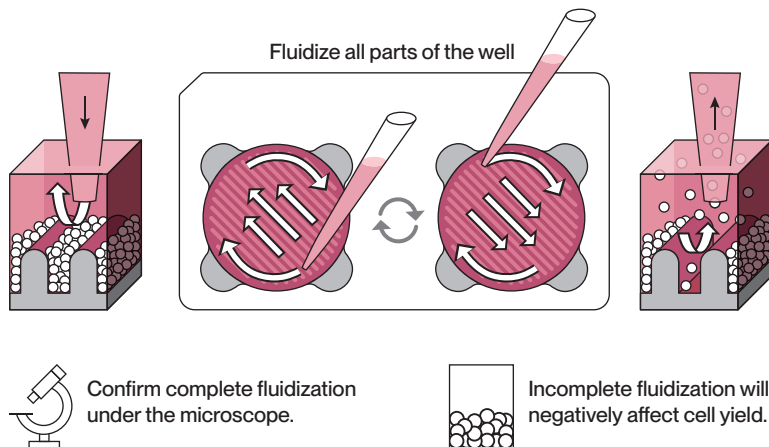
# CELL HARVEST: SUSPENSION CELLS

## 1 Define your suspension volume:

- **24-well:** Aspirate at the 1.0 or 3.0 mL SureShelves™.
- **1-well:** Aspirate at the A, B, or C SureShelves™.



## 2 Pipette and fluidize all parts of the well to generate a single-cell suspension. Fluidize along the grooves; the plate can be tilted toward you during the harvest step and grooves can be rinsed to dislodge cells.



## 3 Collect the cell suspension for downstream processing, analysis, or seeding according to your application-specific workflow.

## SCALING WITH EXPANSIFY™

Process translation between formats can be performed by normalizing key culture parameters to the available growth surface area or by applying the appropriate device scaling factor.

### Example: Scaling from the Expansify™ 24-well plate to the Expansify™ 1-well plate

#### 1. Determine the device scaling factor

- Expansify™ 24-well plate growth surface area: **2 cm<sup>2</sup> per well**
- Expansify™ 1-well plate growth surface area: **70 cm<sup>2</sup>**
- Scaling factor:  $70 \div 2 = 35$

#### 2. Determine the equivalent seeding density

To maintain the same seeding density across formats, scale the cell input according to growth surface area. For example, if one 24-well plate is seeded with **50,000 cells per well** (equivalent to **25,000 cells/cm<sup>2</sup>**), the corresponding 1-well plate should be seeded with:

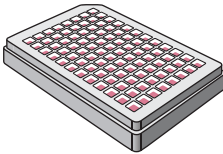
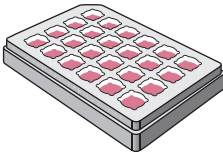
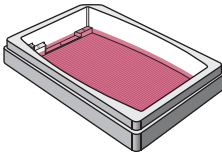
$$25,000 \text{ cells/cm}^2 \times 70 \text{ cm}^2 = 1.75 \times 10^6 \text{ cells}$$

This maintains equivalent cell density relative to the available growth surface area in both formats.

#### 3. Determine media volumes and feeding requirements

Initial fill volumes, media exchange volumes, and feeding schedules should be scaled according to device size and scaling factor, while accounting for culture density, growth rate, and metabolic demand. The Expansify™ 1-well plate has a maximum fill volume of **100 mL**, which corresponds to approximately **3 mL per well** in the 24-well plate based on the 35× surface area scaling factor.

**Note:** Although the Expansify™ 24-well plate can accommodate up to 4.5 mL per well, scaling this volume directly to the 1-well plate would exceed the 100 mL maximum fill volume. When using higher 24-well fill volumes, media volume and feeding strategy should be adjusted based on the specific experiment, cell type, and metabolic demand.

| Style                        | 96-well                                                                            | 24-well                                                                            | 1-well                                                                             |
|------------------------------|------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|
| Product                      |  |  |  |
| Cell yield<br>(e.g. HEK293T) | 3 million/well                                                                     | 20 million/well                                                                    | 500 million                                                                        |
| Surface area                 | 0.55 cm <sup>2</sup> /well                                                         | 2 cm <sup>2</sup> /well                                                            | 70 cm <sup>2</sup>                                                                 |
| Max fill volume              | 0.55 mL                                                                            | 4.5 mL                                                                             | 100 mL                                                                             |

## WHAT CHANGES AT THE BENCH

≈ 24×  Replace up to **24** T-75 flasks with **one** 24-well plate (single lid).  
1× 

≈ 15×  Replace up to **15** T-225 flasks with **one** 1-well plate (single lid).  
1× 

 Rapid aspiration and media dispensing across all 24 wells in a few passes using SureShelves™ in minutes.

 **Parallelization:** run up to 24 T-75-equivalents at once—ideal for replicates, condition testing, or DOE studies.

 **Free your incubator space:** swap a whole shelf of flasks for one ANSI/SLAS-format plate.

 **Consistent high density:** frequent, small-volume media refreshes maintain nutrients and remove waste right when cells need it.

## RECOMMENDED PIPETTE USE WITH EXPANSIFY™

| Pipette tip MUST be fully seated at the back of the SureShelves™                                                 | <b>Aspiration</b><br>at any SureShelves™ | <b>Media addition</b><br>slow, steady rate of ~0.5 mL/s | <b>Media sampling</b><br>at any SureShelves™ | <b>Harvesting</b><br>orient pipette motion parallel to the grooves |
|------------------------------------------------------------------------------------------------------------------|------------------------------------------|---------------------------------------------------------|----------------------------------------------|--------------------------------------------------------------------|
| <br><i>Pasteur pipette</i>      | ✓                                        |                                                         |                                              |                                                                    |
| <br><i>Micropipette</i>        | ✓                                        | ✓                                                       | ✓                                            | ✓                                                                  |
| <br><i>Serological pipette</i> | 1-well only                              | ✓                                                       | ✓                                            | ✓                                                                  |

✓ = 24-well + 1-well



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[www.xdemics.com](http://www.xdemics.com)

