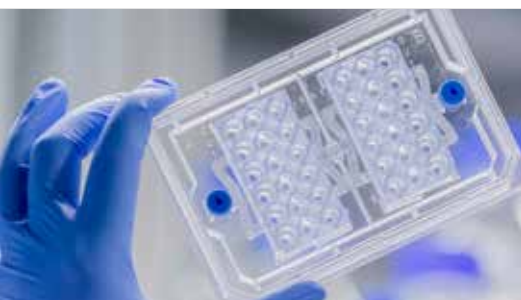



Advanced in vitro exposure systems



Cloud  AX12
Lung-on-Chip

VITROCELL® Cloud AX12 Lung-on-Chip

Aerosol exposure for breathing ^{AX}Lung-on-Chip in close cooperation with AlveoliX

Chip-based exposure of cell cultures

The VITROCELL® Cloud Alpha AX12 is our newest innovation in the Cloud family and presents a great leap forward in automated exposure of cell cultures with breathing function. It combines highly efficient testing with ease of use. The development is based on the well-known and frequently published VITROCELL® Cloud formats (6-, 12-, 24- and 96-well). Its functionality enables fully automated processes with an all-in-one control unit. Everyday experiments at the Air/Liquid Interface have never been easier.

It is suitable for the nebulization of solutions and suspensions. Fields of application are, but are not limited to, screening of inhaled drugs and toxicity testing of inhaled substances such as chemicals, nanoparticles and airborne pathogens.

Air/Liquid Interface

In response to the scientific need to expose in physiologically relevant conditions, the VITROCELL® Cloud Alpha exposure device has been specifically designed to enable direct exposure of cells or tissue at the Air/Liquid Interface. Here, the cell cultures are not covered with media in contrast to submerged conditions, which cause an undesired interaction of the formerly airborne substances with the culture media.

Cells cultivated on the membrane are exposed at the Air/Liquid Interface so that the test substances directly come in contact with the cells as aerosols. This approach allows for more in vivo like and lung-relevant cell response than if exposed to submerged conditions.

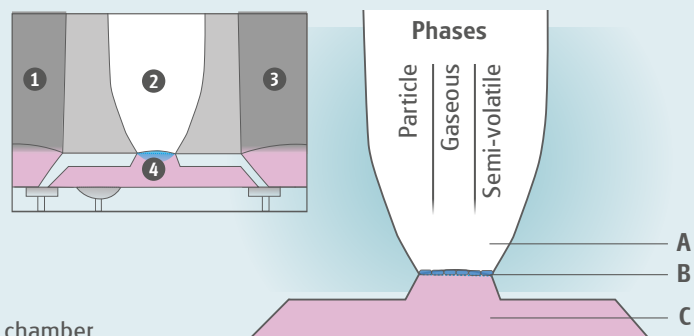


Vitrocell® Cloud Alpha AX12 – developed in close cooperation with AlveoliX.

Air/Liquid Cultivation and Exposure

- A Direct and controlled exposure of test atmosphere to cells
- B Cells on ultrathin ^{AX}membrane
- C Media below cells

(1) Inlet well, (2) Cell well, (3) Outlet well, (4) Fluidic chamber



^{AX}Lung-on-Chip System

The ^{AX}Lung-on-Chip System allows in vitro modeling in outmost physiological conditions by replicating the essential key features of the lung microenvironment.

- Ultrathin, porous, and elastic support for cell growth
- Breathing motion
- Air/Liquid Interface
- 3D tissues (multiple co-cultures)

The combination of the breathing-induced cyclic stretch and the optimized ultrathin cell culture substrate (AX membrane) preserves lung cells' functionality and phenotype. The technology provides answers to fundamental questions about lung biomechanics and cell biology. Furthermore, it is a powerful tool to recreate healthy, diseased, and personalized in vitro models, which can be implemented in drug development to test molecule efficacy and safety, and in toxicity assessment of e. g. pollutants, chemicals and consumer goods. This will hopefully lead to more animal free product development in the future.



AlveoliX ^{AX}Lung-on-Chip System

^{AX}Lung-on-Chip System components

Aside the AX12, the system is made up of three further components: two pneumatic controllers, ^{AX}Breather and ^{AX}Exchanger, and the ^{AX}Dock which is the interface between these two devices and the AX12.

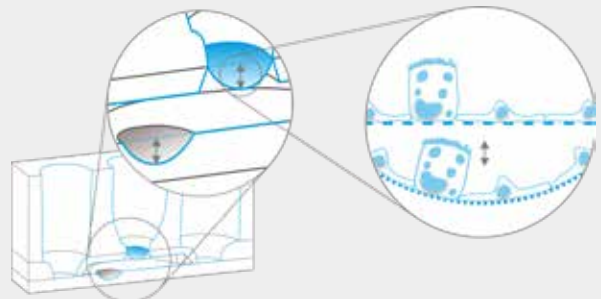
^{AX}Breather The ^{AX}Breather controls the deflection of the micro-diaphragm inside the AX12, recreating the 3D cyclic breathing motion.

^{AX}Dock The ^{AX}Dock connects the AX12 with the ^{AX}Breather and the ^{AX}Exchanger creating an air-tight sealing for the pneumatic actuation.

^{AX}Exchanger The ^{AX}Exchanger enables a fluid flow for initial chip filling and medium exchange by pneumatically controlling the opening and closing of the valves inside the AX12.

AX12 The AX12 is the cell culture consumable and is based on a 96-well plate format. It consists of two chips supported by a plate and includes a total of 12 independent wells.

AlveoliX 3D Breathing concept



A nature-inspired concept is used to recreate the breathing motion. The ultrathin ^{AX}membrane (blue) is deflected by applying negative pressure inside the basal chip chamber through an integrated micro-diaphragm (grey).

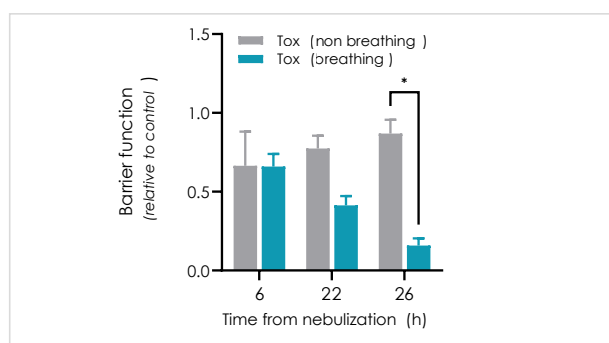
Aerosol Exposure and Lung-on-Chip

Ideal for small quantities of test substance

The VITROCELL® Cloud Alpha AX12 is designed for small nebulization volumes and has very high deposition efficiency. The recommended nebulization volume is 300 µl. Therefore, the device is particularly suitable for testing materials even when quantities are limited.

Dosimetry using Quartz Crystal Microbalance (QCM)

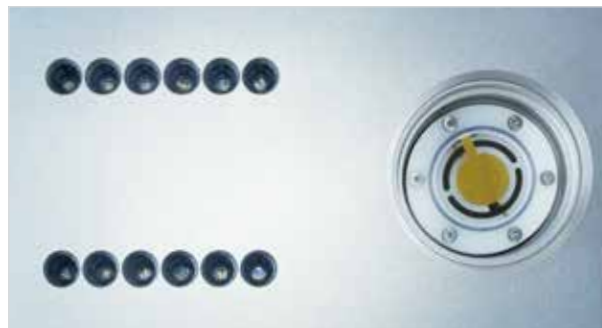
To assess the delivered dose, a QCM sensor is integrated into the Cloud Alpha AX12 exposure module. The microbalance can measure the deposited mass at a resolution of 10 ng/cm² per second. Results are reported online by the VITROCELL® Monitor software. Data is presented in graphs and stored in MS Excel®.



Time-course barrier function of lung epithelial cells upon nebulization with nanoparticles in breathing and non-breathing conditions. The delivered dose is equivalent to the lifetime exposure accumulated by a worker (45 years of work).

Key Features:

- Low nebulization volumes: 300 µl per exposure
- High deposition efficiency
- Easy handling with no external air-flow required
- Air/Liquid Interface exposure
- Breathing Lung-on-Chip system
- Porous membrane allowing cell-cell interaction
- Increased cell sensitivity in physiological conditions



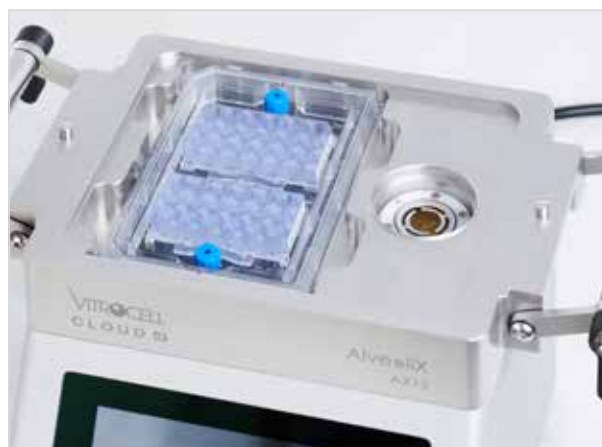
The Cloud Alpha AX12 is equipped with a microbalance sensor.

Exposure combined with 3D breathing motion

Lung epithelial cells cultured on-chip exhibit robust barrier functionality in breathing conditions to the long term. When exposed to nebulized molecules at the Air/Liquid interface, cells demonstrated an increased sensitivity to airborne hazards, such as nanoparticles or known inhaled irritants, in breathing conditions. By simulating the in vivo situation, the Cloud Alpha AX12 in combination with the ^{AX}Lung-on-Chip enables more predictive toxicological assessment, inhalation-derived disease modeling, and potential evaluation of inhaled drugs.

Choice of four types of nebulizers

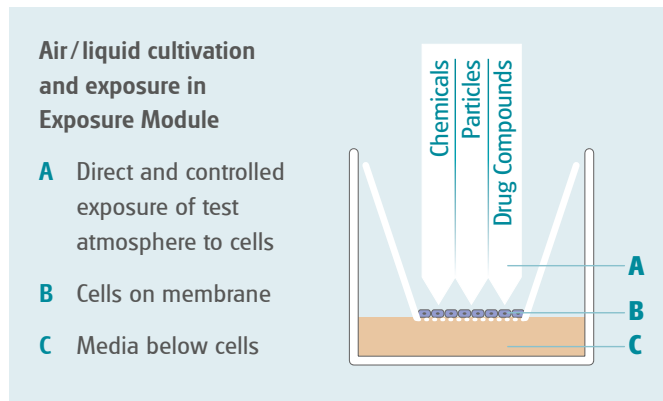
The system comes with a choice of 4 types of nebulizers having droplet MMAD ranges of 2.5 – 4.0 µm, 2.5 – 6.0 µm, 4.0 – 6.0 µm and 9.0 – 12.0 µm.



Cloud Alpha AX12 Base Module with AX12 and QCM

VITROCELL® Exposure Systems for Inhalation Toxicology

Direct Exposure Technology at Air/Liquid Interface



In response to the scientific need to expose in physiologically relevant conditions, VITROCELL® exposure modules have been specifically designed and engineered to enable direct exposure of mammalian cells or tissue at the Air/Liquid Interface where the cell systems are not covered with culture medium.

Researchers can thus use all cell types cultivated on microporous membranes.

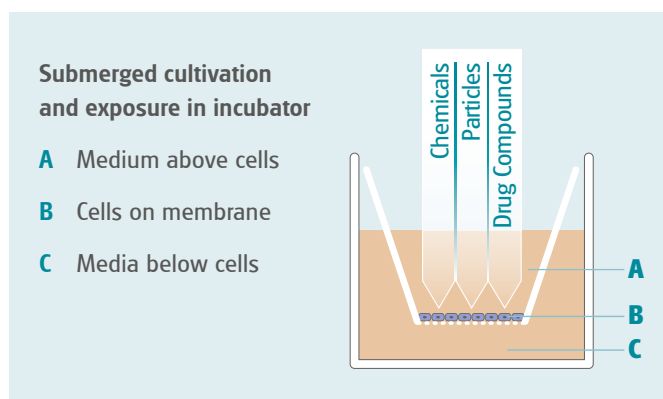
This approach allows for more credible and authentic results than by submerged exposure due to a closer replication of the human physiology.

The advantages

- No losses
- No dissolution
- No reaction of constituents with culture medium
- High sensitivity



Disadvantages of submerged exposure



The exposure of mammalian cells or tissues to airborne substances is frequently performed under submerged conditions. In doing so, the test substances are dosed into the culture medium. This procedure results in an undesired interaction of the formerly airborne substances with the medium, causing limitations for authentic analysis.

Therefore VITROCELL® recommends the air/liquid interface exposure technology.

Exposure Principle

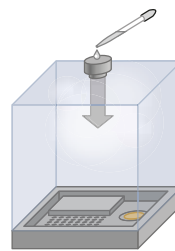
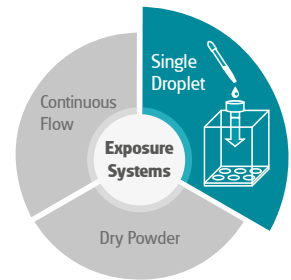
Single Droplet Sedimentation (Cloud Exposure)

Single Droplet Sedimentation Systems are specifically designed for dose-controlled and spatially uniform deposition of liquid aerosols on cells. Test substances are chemicals or particles brought into suspension with e.g. PBS.

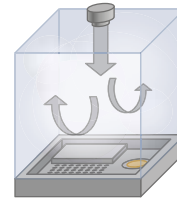
The aerosol is applied for a short time of approx. 3–6 minutes.

This method is well suited for scarce and expensive materials, such as new drug candidates or particle samples from the environment.

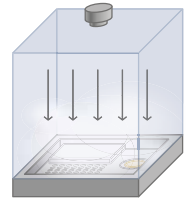
Nebulization volumes range from 15 to 300 µl. Aerosolization is performed directly into the cell culture exposure chamber. Aerosolization can be repeated several times to obtain a dose-response profile.



Phase 1
Emission of
Cloud



Phase 2
Homogeneous
Mixing



Phase 3
Gravitational
Settling

Main application areas



- Particles/nanoparticles in suspensions
- Pharmaceutical compounds/liquids
- Chemicals
- Virus research

Cloud Exposure Systems



Cloud Alpha 12



Cloud Alpha MAX



Cloud Alpha MOVE



Cloud Alpha TRIO

About VITROCELL®

VITROCELL® exclusively concentrates on the developing, producing, installing, training and servicing of advanced *in vitro* exposure systems.

The VITROCELL® Systems' team is driven by their vision for new in-vitro standards through state-of-the-art technology, highly qualified workmanship and absolute client dedication. VITROCELL® has successfully collaborated with clients from leading research institutes, contract research organizations, regulatory authorities or industrial laboratories across the world. Working with our team experts, all modules have been tailored to create durable and complete turnkey-systems for *in vitro* inhalation toxicology. Gases, environmental atmospheres, nano particles and complex mixtures are analyzed on lung cells at the air/liquid interface using these systems. VITROCELL® technologies are also applicable to solutions for skin research.

Over a decade of devotion to research in this specific field has given our team of design & precision manufacturing specialists the opportunity to mentor highly diversified and complex projects from conception to completion. We strive to become a constructive member of each research team, providing support when it is needed, advice when it is required and modules of the highest quality, which are even polished by hand before leaving here to be integrated into your workspace. Every piece of our German engineered equipment is manufactured to the highest of standards – yours.

For more information please scan the QR-Code:



VITROCELL® Systems GmbH
Fabrik Sonntag 3
79183 Waldkirch
Germany

Tel. +49 7681 497 79-50
Fax +49 7681 497 79-79
Email: info@vitrocell.com
www.vitrocell.com

