Protocol | SciKit

Kit components

The Scinora media and supplement Kit (SciKit) has been developed to support sustainable growth for various vertebrate cell lines as well as primary cells derived from animal tissue. It combines the experience in media development for many different cell types of more than 10 years.

All media formulations within this kit are chemically defined and free of any animal-derived components or proteins/peptides. All formulations have unique compositions and by combination, different cell types can be cultured. All media are ready-to-use and contain stable glutamine (L-Ala-L-Gln). No further supplementation is needed Below, the media within the SciKit are listed in a brief summary:

Medium	Description
Medium A Blood cell-specific	Developed for blood-derived cell lines, keratinocytes, hepatoma cells and stem cells. Rich in supporting vitamins as well as co-factors. Osmolality: 270 – 290 mOsmol·kg^{-1}
Medium B Cancer cell-specific	Developed for long-term cultivation of cancer cell lines and exosome production. Rich in nutrients. Suitable for all animal cell lines with high demand for nutrients. Osmolality: 300 – 320 mOsmol·kg^{-1}
Medium C Physiological formulation	Physiological medium including various plasma metabolites. Suitable for co-cultivation and various primary cell lines. Osmolality: 270 – 290 mOsmol·kg^{-1}
Medium D Tissue cell formulation	Developed for long-term cultivation of normal tissue cells and standard cell lines such as HEK293 and HeLa. Osmolality: 300 – 320 mOsmol·kg^{-1}

The supplements supplied contain animal-free protein mixtures in a defined way (SuppA & SuppB) or complex manner (XplaceH).

The supplements are delivered as lyophilized solid and need to be reconstituted in sterile water or medium first. Once reconstituted in 1.0 ml sterile water, aliquots can be frozen and stored at -20°C for 6 months.

Supplement	Description
SuppA Growth factor mix	Chemically defined growth factor mixture to support cell growth for various cell types.
SuppB Hormone mix	Chemically defined hormone mixture to support cell growth and function for various cell types.
XplaceH Liver cell line-derived supplement	Protein-only supplement to improve growth and attachment in chemically defined media. Complex formulation containing proteins for ECM, growth factors, transport proteins and protease inhibitors.

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XplaceH can be used as coating agent. Here, the reconstituted XplaceH (10 mg/ml) is diluted to 0.1 - 1.0 mg/ml in PBS 1x or medium with 25 – 50 µl/cm² for 30 – 60 minutes at 37 °C. Optionally if using medium, the cells can be added to the coating solution directly by diluting with additional medium. Coating is not necessary, but occasionally beneficial.

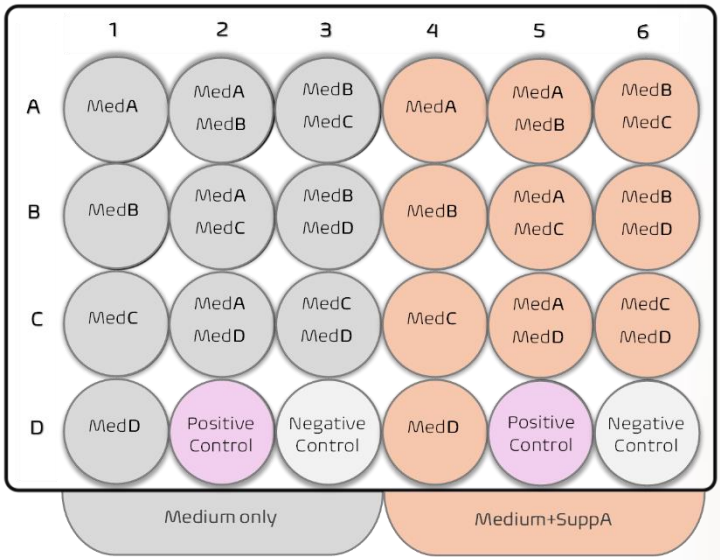
Initial screening | Setup

The initial DoE combinations are performed in small cavities such as 12-well to 48-well plates. We do not recommend 96-well plates based on our experience regarding reproducibility.

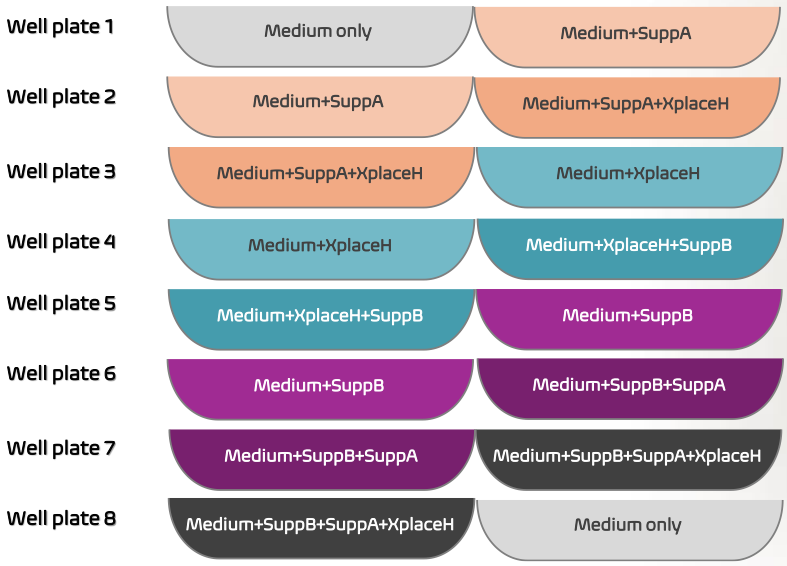
At-a-glance, the first screen involves the seeding of cells in standard growth medium at 2000 - 5000 cells/cm² (*depending on cell size*). At day 1, the medium is removed and the cells are washed twice with PBS 1x.

For cell lines growing in suspension, the seeding density should be at 0.5 - 1.0·10⁶ cells/ml. Here, the media combinations are prepared prior to the addition of the cells in standard medium. More specifically, prepare 1.8 - 1.9 ml medium per well in a 24-well plate and add 100 – 200 µl cell suspension with 5.0 – 10.0·10⁶ cells/ml in fresh standard medium without FCS.

In a 24-well plate, the following setup can be used (*max 2 ml medium/well; Positive control: standard growth medium; Negative control: standard growth medium without FCS*):



Here, the combinations at simple 50 %:50 % mixtures of the respective media. As a first screen with limited number of cells and plates, we recommend to alternately add the different supplements at 1x concentration (1 µl/ml) according to the scheme below:



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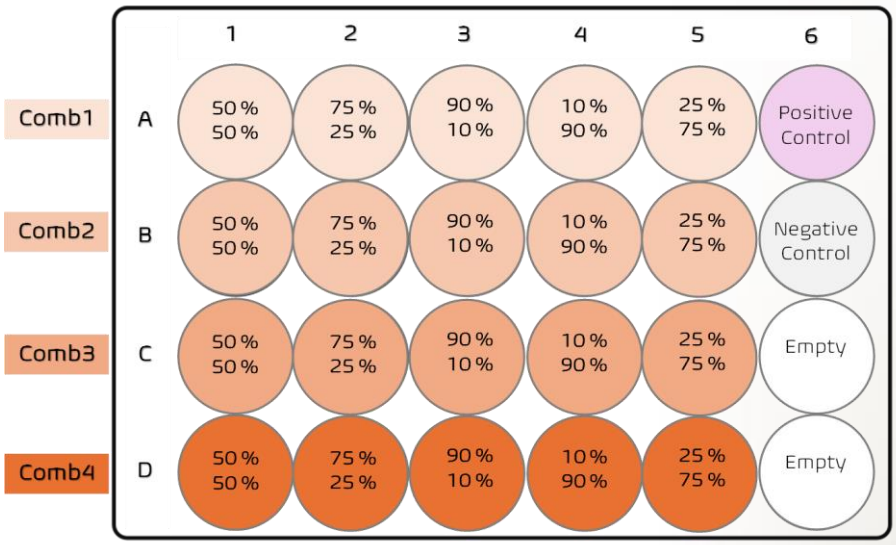
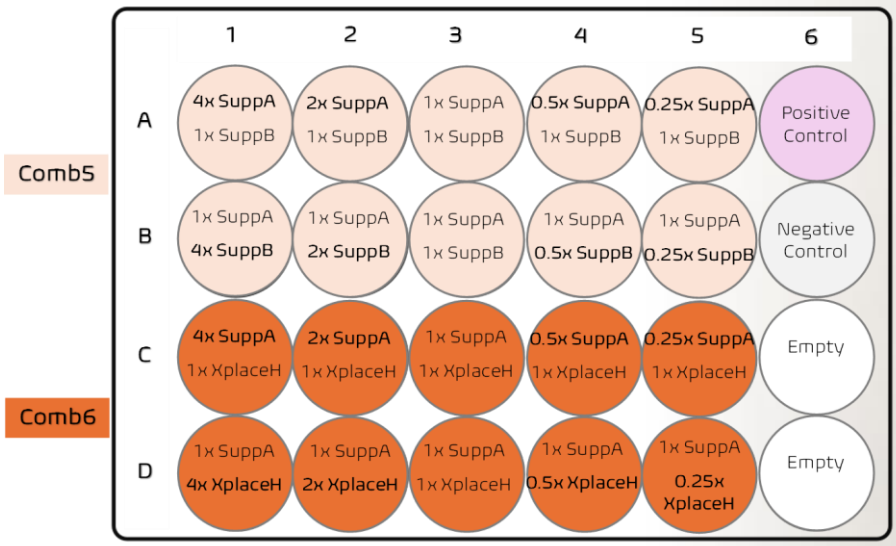
Here, the respective supplement is tested in every media combination in two technical replicates with direct comparison as well as varying one variable only. In general, it is recommended to perform at least two technical replicates as well as two biological replicates. For an initial screen, one biological replicate suits the purpose.

If desired and to further optimize the media composition, perform a second screen by varying the media ratios (75 %:25 % to 90 %:10 %) as well as the supplement additions (4x, 2x 1x, 0.5x 0.25x) prior to the adaptation process. In this case, use the four (4) best combinations and vary the ratios according to the scheme below. Again, the cells are seeded in standard growth medium at 2000 - 5000 cells/cm² and at day 1 the medium is removed including washing of the cells twice with PBS 1x. Alternatively for suspension cell lines, perform the process described above.



Subsequently, the supplement combination is optimized according to the following pattern below:



Here, the supplement concentration are varied the achieve the optimal conditions for the desired purpose. In a subsequent step, the successful combinations are used for direct or gradual adaptation in larger cultivation vessels.

In every case, Scinora is assisting you throughout the selection process to optimise the specific and optimal workflow.

Adaptation process

In following, the steps for a general medium adaptation is descripted (*SFM: serum-free medium formulation after screening; StdM: Standard medium*):

Step	Description
A *Direct adaptation*	- Add 100-200 µl SFM per cm^2 in a desired vessel (e.g. T-75 flask); - Thaw a vial of cells and directly resuspend them into the SFM; - Centrifugation the cell suspension at 300·g, 3 minutes, room temperature; - Resuspend in the SFM and transfer the cells into the vessel at 10^3-$5{\cdot}10^3$ cells per cm^2. - Incubate at desired temperature, optionally with 5 % CO_2, for at least 48 – 72 h or towards 80 % confluency. Change the medium after 3 to 4 days if needed; - In parallel, cultivate the cells in StdM. Proceed with passaging (step **B**).
B *Passaging*	- Remove used medium | Wash with DPBS w/o Ca^{2+}/Mg^{2+} | Dissociate cells; - Centrifugation the cell suspension at 300·g, 3 minutes, room temperature; - Resuspend cell pellet in medium and cell counting; Transfer cell suspension to desired vessel with 100-200 µl medium as well as 10^3-$5{\cdot}10^3$ cells per cm^2, respectively; - Repeat passaging every 4-7 days or change medium after 2-4 days.
C	Adaption is successful after achieving at least 4 continuous passages from SFM.

Alternatively, a sequential adaptation by serum weaning can be applied. Contact us for further information or troubleshooting.

Notes | Troubleshooting

Additional Supplements	Beneficial supplements can be added for specific cell types. In general, the kit incorporates ready-to-use solutions for multiple mammalian cell lines and needs minor supplementation only. Other cell specific factors such as TGF-β, PDGF or KGF/FGF7 are maybe necessary.
Adherence	Depending on cell types and the cell line used there is no pre-coating of vessels needed. These preferences have to be further tested by the customer. Some cell types forms spheroids or low-level clustered cell suspensions, which are capable to grow similar or better than on adherent mode. As an alternative, add additional $CaCl_2$ (sterile; up to 4 mM).
Coatings	Laminin- or collagen-based coatings are compatible, which strongly depend on cell type and cell line. Please use manufacturer's protocol and iteratively optimize the conditions. In general, XplaceH can serve as a coating. Here, increase the coating period and/or the concentration optionally in combination of subsequent washing steps with PBS 1x. Alternatively, the concentration of XplaceH in the medium can be increased up to 50 mg/L to ensure an improved performance.
Poor adaptation performance	If no cell growth is observed for several days, contact us first for further support or add known growth factors for your specific cell type in higher or even lower concentrations. In addition, keep the batch-to-batch variations of the growth factors in mind and test different batches/suppliers.

Available products

Art. No.	Components
100501S	SciKit | 4x 125 ml Media | 1x SuppA (S) | 1x SuppB (S)| 1x XplaceH 10 mg
100501M	SciKit | 4x 500 ml Media | 2x SuppA (S) | 2x SuppB (S) | 2x XplaceH 10 mg
100501L	SciKit | 4x 1000 ml Media | 1x SuppA (L) | 1x SuppB (L) | 1x XplaceH 50 mg
108102-10MG	**XplaceH** | Human liver cell line-derived supplement | lyophilised formulation | for 500 ml – 2 L
108102-50MG	**XplaceH** | Human liver cell line-derived supplement | lyophilised formulation | for 2.5 – 10 L

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