# MyoCult™ Differentiation Kit (Human)

**Medium for the differentiation of human skeletal muscle progenitor cells (myoblasts) into myotubes**

Catalog #05965 1 Kit

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## Product Description

MyoCult™ Differentiation Kit (Human) consists of MyoCult™ Differentiation Medium and Animal Component-Free (ACF) Cell Attachment Substrate. MyoCult™ Differentiation Medium is a serum-based medium specifically formulated for the in vitro differentiation of human skeletal muscle progenitor cells (myoblasts) into myotubes. This medium is suitable for the differentiation of myogenic progenitor cells previously culture-expanded in serum-free medium (e.g. using MyoCult™ -SF Expansion Supplement Kit [Human], Catalog #05980). MyoCult™ Differentiation Medium should be used in conjunction with ACF Cell Attachment Substrate, which has been specifically tested for optimal cell adherence when cells are cultured with MyoCult™ Differentiation Medium.

## Product Information

The following components are sold as a complete kit (Catalog #05965) and are not available for individual sale.

| COMPONENT NAME | COMPONENT # | SIZE | STORAGE | SHELF LIFE |
|---|---|---|---|---|
| MyoCult™ Differentiation Basal Medium (Human) | 05966 | 190 mL | Store at 2 - 8°C. | Stable for 11 months from date of manufacture (MFG) on label. |
| MyoCult™ Differentiation 20X Supplement (Human) | 05967 | 10 mL | Store at -20°C. | Stable until expiry date (EXP) on label. |
| Animal Component-Free (ACF) Cell Attachment Substrate | 07130 | 1 mL | Store at 2 - 8°C | Stable until expiry date (EXP) on label. |

## Preparation of Reagents and Materials

### MyoCult™ Differentiation Medium

Use sterile technique to prepare MyoCult™ Differentiation Medium (Basal Medium + 20X Supplement). The following example is for preparing 200 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw MyoCult™ Differentiation 20X Supplement at room temperature (15 - 25°C) or at 2 - 8°C overnight. Mix thoroughly.

   NOTE: Once thawed, use immediately or aliquot and store at -20°C. Do not exceed expiry date as indicated on the label. After thawing the aliquots, use immediately. Do not re-freeze.
2. Add 10 mL of 20X Supplement to 190 mL of Basal Medium. Mix thoroughly.

   NOTE: If not used immediately, store MyoCult™ Differentiation Medium at 2 - 8°C for up to 1 month. Do not exceed the shelf life of the individual components.

### Coating Cultureware with Animal Component-Free (ACF) Cell Attachment Substrate

Use sterile technique when coating cultureware with ACF Cell Attachment Substrate.

NOTE: Use only tissue culture-treated cultureware.

1. Dilute ACF Cell Attachment Substrate 1 in 300 in D-PBS (Without Ca++ and Mg++; PBS, Catalog #37350). For example, add 20 µL of ACF Cell Attachment Substrate to 5.98 mL of PBS.
2. Gently mix the diluted ACF Cell Attachment Substrate. Do not vortex.

3. Immediately use the diluted ACF Cell Attachment Substrate solution to coat cultureware. Refer to Table 1 for recommended coating volumes.

**Table 1. Volumes Recommended for Coating Cultureware with ACF Cell Attachment Substrate**

| CULTUREWARE | VOLUME OF DILUTED ACF CELL ATTACHMENT SUBSTRATE |
|---|---|
| 6-well plate | 1 mL/well |
| T-25 $cm^2$ flask | 2.5 mL/flask |
| T-75 $cm^2$ flask | 6 mL/flask |

4. Gently rock the cultureware back and forth to spread the ACF Cell Attachment Substrate solution evenly across the surface of the cultureware.
5. Incubate at room temperature (15 - 25°C) for at least 2 hours before use. Do not let the ACF Cell Attachment Substrate solution evaporate.

   NOTE: If not used immediately, the cultureware must be sealed to prevent evaporation of the ACF Cell Attachment Substrate solution (e.g. with Parafilm®). Sealed cultureware can be stored at 2 - 8°C for up to 3 days after coating. Allow stored coated cultureware to come to room temperature (15 - 25°C) for 30 minutes before proceeding to the next step.
6. Gently tilt the cultureware onto one side and allow the excess ACF Cell Attachment Substrate solution to collect at the edge. Remove the excess solution using a serological pipette or by aspiration. Ensure that the coated surface is not scratched.
7. Wash the cultureware once using PBS (e.g. use 2 mL/well if using a 6-well plate).
8. Aspirate wash solution when cells are ready to be plated.

## Directions for Use

The following protocol is for differentiation of myogenic progenitor cells in 6-well plates (e.g. Catalog #38015). If using alternative cultureware, adjust volumes accordingly.

1. Coat desired wells with ACF Cell Attachment Substrate (see Preparation of Reagents and Materials).
2. Add 2 mL of growth medium (e.g. using MyoCult™ -SF Expansion Supplement Kit [Human], Catalog #05980) to each coated well.
3. Plate human myogenic progenitor cells at 1.5 x 10^5 cells/well. Incubate at 37°C and 5% $CO_2$.
4. Once cells reach a confluency of 85 - 95% (approximately 24 - 48 hours), remove growth medium. Wash cells once with 2 mL of warm PBS per well.
5. Add 2 mL of MyoCult™ Differentiation Medium to each well. Incubate at 37°C and 5% $CO_2$.

   NOTE: Myotube formation should begin after 3 - 5 days (depending on passage number). For culture times longer than 5 days, replace medium every 3 - 4 days.

## Related Products

For related products, including antibodies, small molecules, and cultureware, visit www.stemcell.com/myogenicworkflow, or contact us at techsupport@stemcell.com.