



## Cardiomyocyte Cellutions Maintenance Medium (Cardiomyocytes Culture Protocol)

### Product Information

<b>Catalog Number</b>	<b>ASM-6016</b>
<b>Handling Instructions</b>	ASM-6016 basal medium is shipped at 4°C and needs to be stored at 4°C upon receipt; at this temperature ASM-6016 basal medium is stable for 1 year post manufacture date. ASM-6016 basal medium is light sensitive; therefore, exposure to light needs to be minimized. ASM-6016-S (growth supplements) is shipped in dry ice and needs to be stored at -20°C upon receipt; at this temperature ASM-6016-S is stable for 1 year post manufacture date.
<b>Quality Control</b>	Medium and supplements undergo sterility testing to assure they are free from bacterial and fungal contamination. The complete medium has been tested and shown to support optimal growth of human cardiomyocytes. In addition, pH, osmolality, endotoxin absence, and other parameters are determined.
<b>Storage Conditions</b>	Store basal medium, reconstituted and complete media in the dark, at 4°C.
<b>Shelf Life</b>	Supplements are stable at -20°C for 1 year Basal medium is stable at 4°C for 1 year Reconstituted medium is stable at 4°C for 30 days Complete medium is stable at 4°C for 1 week

### Materials Required

Materials	Equipment
<ul style="list-style-type: none"><li>• Cardiomyocytes (ASE-5062)</li><li>• Cardiomyocyte Cellutions Maintenance Medium (ASM-6016)</li><li>• 1X PBS (Ca<sup>2+</sup>/Mg<sup>2+</sup>+free)</li><li>• Collagen coating solution (150µg/mL)</li><li>• TrypLE Express</li><li>• Dimethyl Sulfoxide (DMSO)</li><li>• TGF-β-1 (1ng/mL)</li><li>• Fetal Bovine Serum (FBS)</li><li>• 6 or 12 well plates</li><li>• 15 or 50ml Tubes</li><li>• Ice</li></ul>	<ul style="list-style-type: none"><li>• Incubator, 37°C/5% CO<sub>2</sub></li><li>• Water Bath 37°C</li><li>• Centrifuge</li><li>• Pipette</li><li>• Hemocytometer</li></ul>

### Protocol

#### 1. Using Cardiomyocytes Cellutions Maintenance Medium

1.1 Thaw and add ASM-6016-S to ASM-6016 basal medium; the reconstituted medium is stable at 4°C for 1 month post reconstitution.

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1.2 Prepare ASM-6016 complete medium in small batches. To the reconstituted ASM-6016 medium, add TGF-beta-1 (not included) to 1 ng/mL, final concentration, yielding ASM-6016 complete medium, which is stable for 1 week at 4°C.

## 2. Immediately Upon Delivery

- 2.1 Remove vial from shipping container to check that it is still frozen.
- 2.2 Transfer frozen vial to liquid nitrogen until you are ready to thaw and begin cell culture.
- 2.3 Prior to thawing cells, make sure all reagents are ready to use.
- 2.4 Coat cell culture vessels with collagen coating solution and incubate at room temperature, 2h.
- 2.5 Aspirate collagen coating.
- 2.6 Wash the culture vessel with PBS.

## 3. Thawing Cells

- 3.1 Perform and maintain all cell culture using aseptic techniques.
- 3.2 Aliquot and warm only media required. Add fresh TGF-β-1.
- 3.3 Thaw cells rapidly and with agitation in FBS.
- 3.4 Centrifuge at 400 x g for 5 minutes. Remove supernatant and resuspend cell pellet in 1-5 mL fresh 37°C warm ASM-6016 medium (with TGF-β-1).
- 3.5 Count cells using a hemocytometer.

## 4. Plating and Maintaining Cells

- 4.1 Place cells into collagen-coated vessel. Seed cells at a minimum density of  $5.0 \times 10^4$  cells/cm<sup>2</sup>.
- 4.2 Leave cells undisturbed for a minimum of 2 days. Feed cells every 2-3 days by completely removing the media, and then adding fresh cardiomyocyte cellutions medium.

*Note: There will be many unattached cells. You may collect these, centrifuge and reseed.*

- 4.3 Observe cells on a daily basis.
- 4.4 Cells should be fed and kept in culture for a minimum of 10 days prior to use.
- 4.5 Cells should be kept confluent for optimal results.