

Cor.At[®]

Cardiomyocytes derived from
Mouse Embryonic Stem Cells

Protocol

Cor.At[®] Cardiomyocytes Removal of Dead Cells

Using the MACS[®] Technology
(Miltenyi Biotec GmbH, Bergisch Gladbach)

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1 Introduction

Cor.At[®] cells are cardiomyocytes derived from transgenic mouse embryonic stem cells. These cells are puromycin resistant and have the green fluorescent protein (GFP) reporter gene driven by a cardiac-specific promoter (α MHC promoter).¹

Cor.At[®] cells are produced through *in vitro* differentiation of mouse embryonic stem (ES) cells and puromycin selection of cardiomyocytes. Through BrdU incorporation assays, Cor.At[®] cells were determined to have limited proliferative capacity similar to primary cells. These highly pure cells (>99% purity) express cardiac-specific Connexin-43, an indication of the ability for electric coupling of these cells, as shown in immunostaining. Patch clamp analysis, as well as micro electrode array (MEA) recordings, demonstrate the normal electrophysiological properties of these cells.

1.1 Cor.At[®] Cardiomyocytes for Pharmacological and Toxicological Screening

Cor.At[®] cardiomyocytes are particularly useful for cell-based *in vitro* assays in pharmacology and toxicology. These cells are ideal for high content screening, as well as, for medium to high throughput screening.

Cor.At[®] cells can be used for:

- Safety pharmacology
- Toxicological analysis of compounds (cardiac-specific cytotoxicity)
- Screening for pharmacological effects
- Drug development
- Molecular and cell biology
- Research & development

1.2 Cor.At[®] Cardiomyocytes Compared to Cell Lines and Primary Cells

Cor.At[®] cardiomyocytes have many advantages over primary cells and cell lines. These cells are highly standardised and are 99.9% pure. The culture and maintenance of Cor.At[®] cardiomyocytes require minimal laboratory time when compared to the culture and maintenance of rat neonatal cells. Reproducible results can be expected for every assay.

These homogeneous cells are:

- Standardised from lot-to-lot
- 99.9% pure and have fully functional cardiac phenotype
- Frozen, stored and thawed with complete recovery of functionality
- An entire *in vitro*-based system

Cor.At[®] cells can be purchased frozen in cryo vials (Catalog # 06101). Cells are cultured for 48 hours to allow them to recover post thaw and should be cultured on **BD BioCoat™ Fibronectin**-coated dishes (Catalog # 354457 for 35 mm dishes or Catalog # 354403 for 60 mm dishes). For coating plates and dishes of alternate sizes, we recommend using **Sigma Fibronectin** from bovine plasma (1 mg/ml solution), Catalog # F-1141

1.3 The principle of the Dead Cell Removal Kit from Miltenyi Biotec GmbH

After thawing of Cor.At cardiomyocytes, approximately 30% to 50% of the recovered cells are usually trypan blue positive. The amount of dead cells in the culture can be significantly reduced to 5 – 10% using the Dead Cell Removal Kit from Miltenyi Biotec GmbH (Bergisch Gladbach, Germany).

This kit contains magnetic micro-beads coupled to annexin V. Annexin V recognizes phosphatidyl serine (PS) that is present only on the intracellular part of the plasma membrane of viable cells. After induction of cell death by apoptotic or necrotic processes, PS is exposed on the extracellular part of the cells. The annexin V-coupled beads bind to the PS and dead cells can be separated from the viable population using the MACS Separator. The non-labeled viable cells are collected in the effluent of the column and can be applied to the desired experiments after an additional centrifugation step.

2 Materials and Reagents

Please read the entire technical manual before beginning the culture of mouse cardiomyocytes. Additional information on stability and storage instructions for the cells can be found in the specific Product Information Sheets (PIS).

2.1 Product Specification

Catalog #	Product	Description	Unit / Format	Storage Conditions
AX-001a-1M AX-001aCL	Cor.At® Cells & Cor.At® CL Cells (Vials)	<ul style="list-style-type: none"> 1 million viable Cor.At® cardiomyocytes > 99.9% pure 	Cryo vial	Liquid nitrogen (6 months) -80°C* (4 weeks)***
AX-CCM250	Cor.At® Media	<ul style="list-style-type: none"> Culture medium for Cor.At® cardiomyocytes 	250 ml bottle	-20°C (6 months)** 4°C* (2 weeks)***
N/A		<ul style="list-style-type: none"> Puromycin stock solution (10 mg/ml) 	1 ml vial	see above
Miltenyi**** 130-042-102	MiniMACS® Separator	<ul style="list-style-type: none"> Magnet for the separation with magnetic MicroBeats 		N/A
Miltenyi**** 130-042-303	MACS® MultiStand	<ul style="list-style-type: none"> Stand for insertion of MACS magnet 		N/A
Miltenyi**** #130-090-101	MACS® Dead Cell Removal MicroBeads	<ul style="list-style-type: none"> Annexin V-coupled MACS® colloidal super-paramagnetic MicroBeats and 20x Binding Buffer 	1 ml Beat solution, 25 ml 20x Binding Buffer	4°C (6 months)**
Miltenyi**** 130-042-201	MACS® Columns MS	<ul style="list-style-type: none"> MS Columns for the selection of human and animal cells 	25 columns	Room temperature

*Refer to TDS for additional information

**Expiry date as stated on label (i.e. 6 months from date of manufacture)

***from date of delivery of product(s)

****Refer to Miltenyi Biotec for more information

2.2 Additional Materials and Reagents

- Vertical laminar flow hood certified for Level I handling of biological materials
- Incubator with humidity and gas control to maintain 37°C and 95% humidity in an atmosphere of 5% CO₂ in air
- 37°C water bath
- Inverted fluorescence microscope with Green Fluorescent Protein (GFP) or Fluorescein Isothiocyanate (FITC) filters (recommended but not required)
- Sterile 50 ml polypropylene (PP) tubes
- Sterile pipette tips
- Ethanol p.a. (99.9% pure)
- Autoclave

3 Safety Instructions

- All materials are intended for *in vitro* Research Use Only
- They are not intended for Diagnostic, Therapeutic or Clinical Use and is not approved for human *in vivo* applications.
- Cor.At[®] cells are genetically modified mouse cells and should be handled according to local directives (Biosafety level 1).
- Cor.At[®] cells can be inactivated by autoclaving at 121°C for 20 minutes.
- Cor.At[®] cardiomyocytes should be cultured in a sterile environment according to good cell culture and good laboratory practices.
- It is highly recommended that gloves and labcoats be worn when handling all reagents as some reagents contain chemicals that may be harmful. Please consult the PIS and Material Safety Data Sheets (MSDS) for additional safety instructions where applicable.

4. Dead Cell Removal Protocol

All steps have to be performed in a sterile environment in a laminar flow hood.

4.1 Preparation of 1x Binding Buffer

- 1) Per vial of at least one million Cor.At® cardiomyocytes, dilute 0.2 ml of 20x Binding Buffer Stock Solution with 3.8 ml of sterile, double distilled water
Maximum capacity of the column for the selection are 10^7 million out of 10^8 million cells.



4.2 Magnetic labeling

- 1) Thaw a vial of Cor.At® cardiomyocytes according to the Technical Manual.
- 2) Determine the total recovery of cells, viability and recovery of viable cells as described in the Cor.At Technical Manual. The amount of r viable Cor.At® cardiomyocytes should be at least 1 million cells to start with the Dead Cell Removal Kit.
- 3) After determination of cell count and viability, centrifuge cells at 200x g for 5 min.
Important: Use swinging bucket rotor for the centrifugation to avoid loss of cells.
- 4) Remove supernatant completely using a slow-running suction pump or a pipette.
- 5) Resuspend the cell pellet in 100 μ l of Dead Cell Removal MicroBeads. Mix carefully and incubate for 15 minutes at room temperature (20–25 °C).
- 6) In the meantime, prepare the MACS MS column as described below.

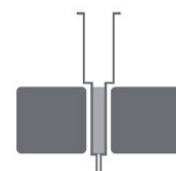


4.3 Preparation of MACS® MS Column

- 1) Apply the magnetic MACS® Separator to the separator stand, sterilize it with 70% ethanol and place it in the laminar flow hood.
- 2) Choose a positive selection column type MS, transfer and open the package of the column under the laminar flow hood (keep it sterile!) and place it in the holder of the MACS® Separator.
- 3) Prepare the column by rinsing it with 500 μ l 1x Binding Buffer (prepared in step 1 of 4.1) and discard the effluent.

4.4 MACS® Separation

- 1) Place a fresh 50 ml tube under the MS column.
- 2) After the 15 min incubation of the cells with the magnetic bead solution, add 500 μ l 1x Binding Buffer to the cell suspension.



- 3) Resuspend carefully and pipette the cell suspension into the column.
- 4) Collect the effluent in the 50 ml tube, which contains the negative, viable cells.
- 5) Rinse the column 4x with 500 μ l of 1x Binding Buffer and collect all effluents in the 50 ml tube.
- 6) Centrifuge collected cells at 200x g for 5 min and resuspend in 500 μ l Cor.At[®] media.
- 7) Determine the total recovery of cells, viability and recovery of viable cells as described in the Cor.At technical manual.
- 8) Proceed with your desired experiment.



5 Limited Use Label License

Cor.At[®] cells, including its derivatives or modifications, are produced in part with technology that is the subject of pending German, U.S. and foreign patent applications and/or patents owned or licensed by Axiogenesis AG (hereinafter "Axiogenesis IP"). The purchase of this product conveys to the buyer the non-exclusive, non-transferable right to use the purchased amount of Cor.At[®] cells and the associated Axiogenesis IP for (i) not-for-profit internal research conducted by the buyer (whether the buyer is an academic or for-profit entity), where "Research" means non-commercial uses or activities which (or the results of which) do not generate revenue, and (ii) certain for-profit activities, including lead discovery, testing and/or research and development of products or potential products that may or may not be, or become, commercially available, quality control of commercial products, and provision of services consisting of these applications to third parties. Axiogenesis AG hereby waives its right to any compensation beyond the price paid by purchaser for Cor.At[®] cells when Cor.At[®] are used for the activities permitted by this license, including the right to any intellectual property or any profits generated by purchaser through purchaser's use, directly or indirectly, of Cor.At[®] cells. Accordingly, Axiogenesis AG will not assert a claim against the buyer of infringement of its relevant patents based upon the manufacture, use or sale of a product (including a therapeutic, clinical diagnostic, vaccine, prophylactic or research product) developed in research by the buyer in which Cor.At[®] cells were employed, provided that neither Cor.At[®] cells nor any of its components were used directly in the manufacture of such product. This limited use license does NOT include the right to resell the product, use Cor.At[®] cells directly in the manufacture of a product or as a component of a product, reverse engineer Cor.At[®] cells or Axiogenesis IP, or to use Axiogenesis IP in any way that is separated from intact Cor.At[®] cells (including the isolation and/or use of integrated genetic constructs that represent, in whole or in part, Axiogenesis IP).

For information on the foregoing patents or patent applications or on purchasing a license to use Axiogenesis IP for purposes other than those permitted above, contact Business Department, Axiogenesis AG, Nattermannalle 1 / S 20, 50829 Cologne, Germany.

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