EasySep™ Human CD56 Positive Selection Kit II

Positive Selection

Catalog #17855

For processing 5 x 10^7 cells from muscle cultures

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Description

Isolate highly purified CD56+ cells from fresh or previously frozen human muscle cultures (myoblasts and fibroblasts) in as little as 24 minutes by immunomagnetic positive selection.

- Fast and easy-to-use
- Up to 95% purity
- No columns required

This kit targets CD56+ cells for positive selection with antibodies recognizing the CD56 surface marker. Desired cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Unwanted cells are simply poured off, while desired cells remain in the tube. Isolated cells are immediately available for downstream applications such as flow cytometry, culture, or DNA/RNA extraction.

- This is the Product Information Sheet (PIS) for isolating CD56+ cells from human muscle cultures. If isolating CD56+ cells from human peripheral blood mononuclear cells (PBMCs), refer to the applicable PIS (Document #DX20216).

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human CD56 Positive Selection Cocktail II	17855C	1 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS. Includes an Fc receptor blocking antibody.
EasySep™ Dextran RapidSpheres™ 50100	50100	1 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A suspension of magnetic particles in water.

PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

Sample Preparation

MUSCLE CULTURES

Muscle cultures can be established from human skeletal muscle tissue using MyoCult™-SF Expansion Supplement Kit (Human; Catalog #05980). For complete instructions, refer to the applicable PIS (Document #DX22313).

Alternatively, human skeletal muscle cultures can be generated using the methods published by Agley CC et al. and Soriano-Arroquia A et al.[1-2]

After preparation, resuspend cells at 5 x 10^6 cells/mL in recommended medium.

PERIPHERAL BLOOD OR LEUKAPHERESIS

If processing peripheral blood or leukapheresis samples, refer to the applicable PIS (Document #DX20216).

Recommended Medium

EasySep™ Buffer (Catalog #20144), or PBS containing 2% FBS and 1 mM EDTA. Medium should be free of Ca++ and Mg++.

Directions for Use – Manual EasySep™ Protocols

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 1 for detailed instructions regarding the manual EasySep™ procedure for each magnet.

Table 1. EasySep™ Human CD56 Positive Selection Kit II Protocol

STEP	INSTRUCTIONS	EasySep™ (Catalog #18000) 
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10^6 cells/mL 0.2 - 1 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)
2	Add Selection Cocktail to sample.	100 µL/mL of sample
	Mix and incubate.	RT for 10 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
4	Add RapidSpheres™ to sample.	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes
5	Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 2.5 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes
6	Pick up the magnet, and in one continuous motion invert the magnet and tube,** pouring off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant
7	Repeat steps as indicated.	Steps 5 and 6, two more times (total of 3 x 3-minute separations)
8	Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube.	Isolated cells are ready for use

RT - room temperature (15 - 25°C)
* Leave the magnet and tube inverted for 2 - 3 seconds, then return upright. Do not shake or blot off any drops that may remain hanging from the mouth of the tube.

Notes and Tips

ASSESSING PURITY

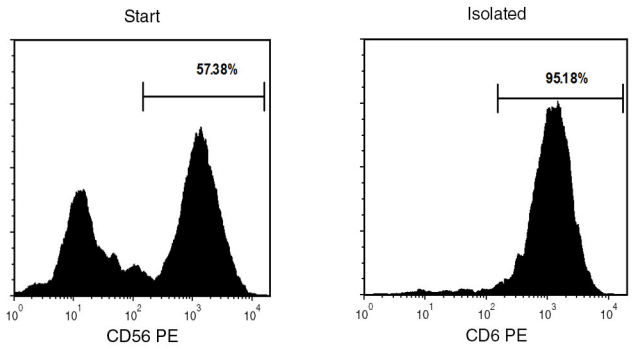
For purity assessment of CD56+ cells by flow cytometry, use one of the following fluorochrome-conjugated antibody clones:

- Anti-Human CD56 Antibody, Clone HDC56 (Catalog #60021; partial blocking), or
- Anti-human CD56 antibody, clone CMSSB (partial blocking), or
- Anti-human CD56 antibody, clone NCAM16.2 (partial blocking)

The following method can also be used:

- Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse Ig (H+L) Antibody, Polyclonal (Catalog #60138).

Data



Starting with muscle cultures at 5 - 10 days after derivation, the CD56+ cell content of the isolated fraction is typically 95 ± 4.3% (mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 57.38% and 95.18%, respectively.

References

1. Agley CC et al. (2015) Isolation and quantitative immunocytochemical characterization of primary myogenic cells and fibroblasts from human skeletal muscle. J Vis Exp (95): 52049.
2. Soriano-Arroquia A et al. (2017) Preparation and culture of myogenic precursor cells/primary myoblasts from skeletal muscle of adult and aged humans. J Vis Exp (120): e55047.