abcam

Product datasheet

ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes ab277612

20 Images

Overview

Product name	ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes
Parental Cell Line	iPSC
Organism	Human
Tested applications	**Suitable for:** ICC, WB, RT-PCR, High throughput screening, Functional Studies
Biosafety level	1
General notes	Introducing the ioSkeletal Myocytes, generated from human induced pluripotent stem cells (iPSCs) using opti-ox, a precise cellular reprogramming technology. Human stem cells, within days, convert into consistent and reliable skeletal myocytes, providing a high-quality human model for research, disease modelling and HTS. ioSkeletal Myocytes demonstrate robust expression of key proteins of myofilaments (Desmin, Titin, Troponin, Myosin Heavy Chain, Dystrophin), coupled with the transition from immature (MYH3 and MYH8) to mature myosin heavy chain isoforms (MYH1) in a time dependent manner. By Day 10 post revival, skeletal myocytes form striated multinucleated myocytes that contract in response to acetylcholine. Human skeletal myocytes are available at scale, easy to culture and ready for experiments within days, providing a reliable model for the study of muscle, neuromuscular and associated metabolic disorders. **In partnership with bit.bio** **Karyotype:** Normal **Seeding Density:** 100,000 cells/cm^2 **Seeding compatibility:** 6-, 12-, 24- and 96-well compatible **Quality control:** Sterility, ICC and gene expression analysis **Research applications:** Muscle research, Neuromuscular junction Research, Metabolic research, Drug development, Genetic screening (e.g. CRISPR screening), Contractions assays *This product is subject to limited use licenses from iPS Academia Japan Inc, TET Systems GmbH, ERS Genomics Limited and Sigma-Aldrich Co. LLC and is developed with Bit Bio patented technology. For full details of the licenses and patents please refer to our **limited use license and patent pages**.*

Properties

Number of cells	Small - $2.5x10^6$ cells/vial; Large - $5x10^6$ cells/vial
Viability	>85%
Cell type	skeletal myocyte
Gender	Male
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituent: 10% DMSO

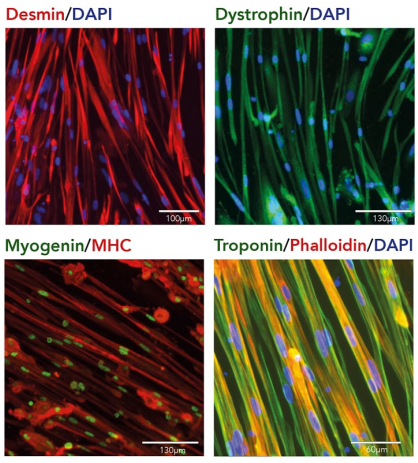
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab277612 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration.
RT-PCR		Use at an assay dependent concentration.
High throughput screening		Use at an assay dependent concentration.
Functional Studies		Use at an assay dependent concentration.

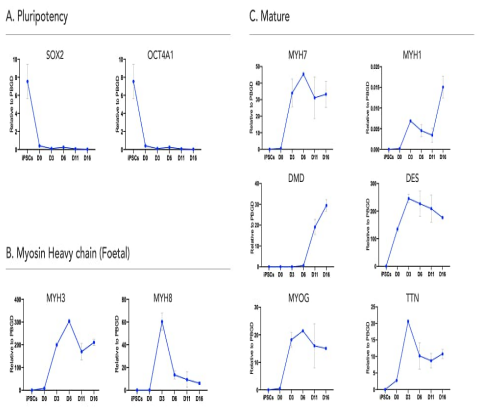
Images



Immunocytochemistry - ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612)

High purity skeletal myocytes express myofilament proteins.

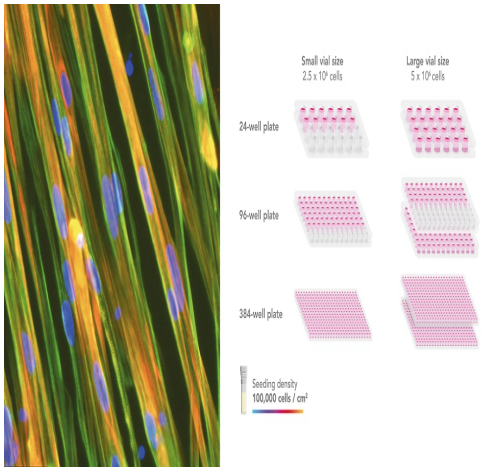
Immunofluorescence staining at day 10 post revival demonstrates robust expression of components of the contractile apparatus such as Desmin (top left), Dystrophin (top right), and Myosin Heavy Chain (bottom left), along with the muscle transcription factor Myogenin (bottom left). Cells also demonstrate expression of Troponin with visible striated fibres and multinucleation (bottom right).



Functional Studies - ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612)

Cells demonstrate timewise gene expression of key myogenic markers.

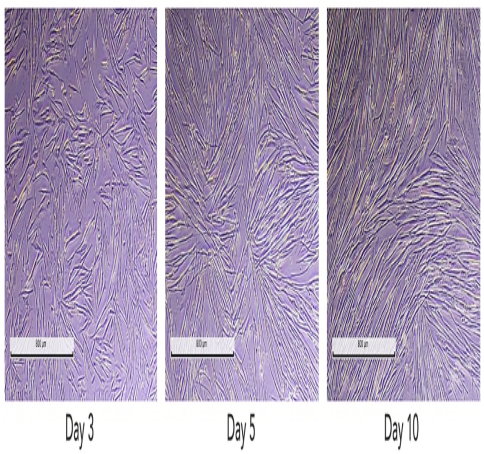
ioSkeletal Myocytes gene expression. Following reprogramming, ioSkeletal Myocytes downregulate expression of pluripotency markers (A), and begin to express myosin heavy chain isoforms MYH3 and MYH8 (B). Through continued culture, ioSkeletal Myocytes demonstrate expression of mature myosin isoforms MYH7 and MYH1, along with DESMIN, DYSTROPHIN, MYOGENIN, and TITIN (C). Gene expression levels assessed by RT-qPCR (data expressed relative to parental iPSC, normalised to PBGD). Data represents day (Dx) post-thaw.



Cell Culture - ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612)

ioSkeletal Myocytes are available in two vial sizes, tailored to suit your experimental needs with minimal waste.

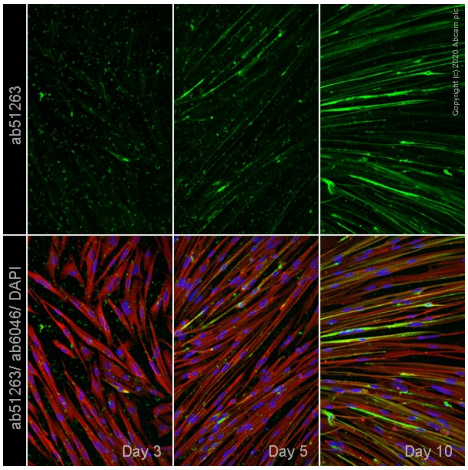
- Recommended seeding density for ioSkeletal Myocytes is 100,000 cells/cm^2.
- One Small vial (2.5 x 10^6 viable cells) can plate a minimum of 0.5 x 24-well plate, 0.75 x 96-well plate, or 1 x 384-well plate.
- One Large vial (5 x 10^6 viable cells) can plate a minimum of 1 x 24-well plate, 1.5 x 96-well plate, or 2 x 384-well plates.



Cell Culture - ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612)

Cells demonstrate classical myocyte morphology.

Day 1 to 10 post-thawing; 4X magnification; scale bar: 800µm



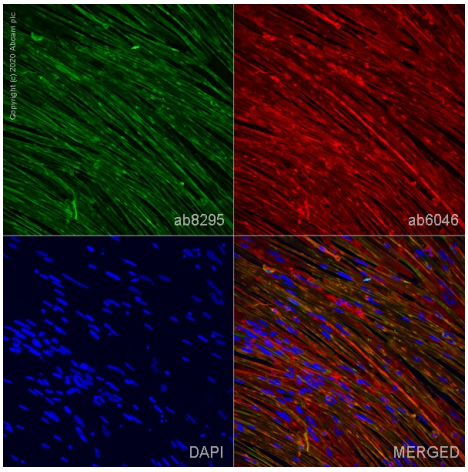
Immunocytochemistry - ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612)

Immunofluorescence staining of Fast Myosin Skeletal Heavy chain using **ab51263** in ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612), which were differentiated for 3 (left panel), 5 (middle panel) and 10 days (right panel) post induction.

The cells were fixed with 100% MeOH (5 min) and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab51263** at 5 µg/mL and **ab6046**, rabbit polyclonal to beta Tubulin, at 1/1000 dilution. Cells were then incubated with **ab150117**, Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150088**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown. Gamma is adjusted to 1.5 in all channels.

The antibody **ab51263** gave comparable results using 4% formaldehyde fixation (10 min).



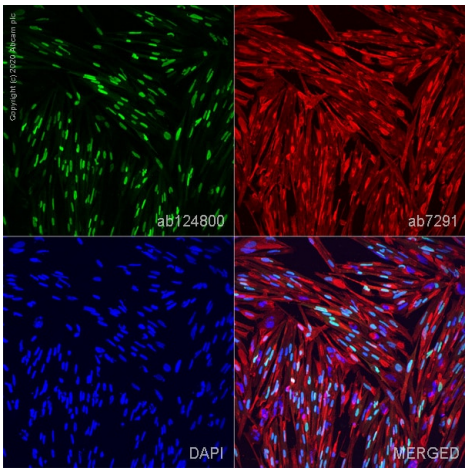
Immunocytochemistry - ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612)

Immunofluorescence staining of Cardiac Troponin T using **ab8295** in ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612), which were differentiated for 10 days post induction.

The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% PBS-Tween for 5 mins and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab8295** at 5 µg/mL and **ab6046**, rabbit polyclonal to beta Tubulin, at 1/1000 dilution. Cells were then incubated with **ab150117**, Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150088**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown. Gamma is adjusted to 1.5 in all channels.

The antibody **ab8295** gave comparable results using MeOH fixation (100%, 5 min).



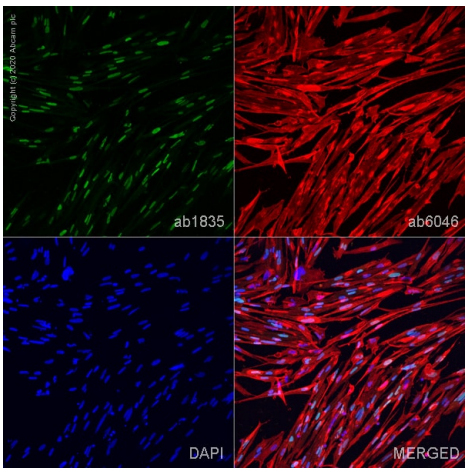
Immunocytochemistry/ Immunofluorescence - ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612)

Immunofluorescence staining of Myogenin using **ab124800** in ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612), which were differentiated for 3 days post induction.

The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% PBS-Tween for 5 mins and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab124800** at 0.5 µg/mL and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin, at 1/1000 dilution. Cells were then incubated with **ab150081**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown. Gamma is adjusted to 1.5 in all channels.

The antibody **ab124800** gave comparable results using MeOH fixation (100%, 5 min).



Immunocytochemistry/ Immunofluorescence - ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612)

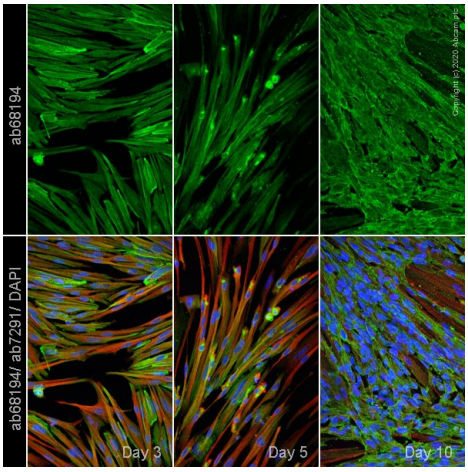
Immunofluorescence staining of Myogenin using **ab1835** in ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612), which were differentiated for 3 days post induction.

The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% PBS-Tween for 5 mins and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab1835** at 1 µg/mL and **ab6046**, rabbit polyclonal to beta Tubulin, at 1/1000 dilution. Cells were then incubated with **ab150117**, Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150088**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS,

Perkin Elmer) and a maximum intensity projection of confocal sections is shown. Gamma is adjusted to 1.5 in all channels.

The antibody **ab1835** gave comparable results using MeOH fixation (100%, 5 min).

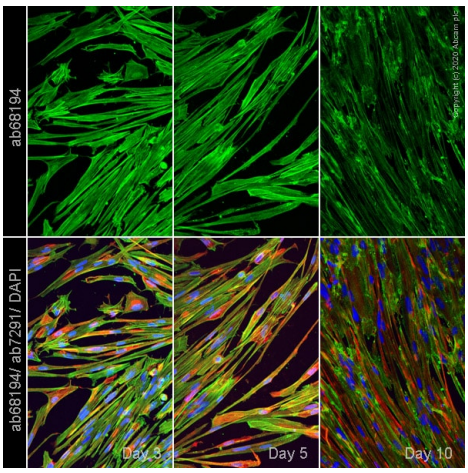


Immunocytochemistry/ Immunofluorescence - ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612)

Immunofluorescence staining of Actinin/ACTN1 using **ab68194** in ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612), which were differentiated for 3 (left panel), 5 (middle panel) and 10 days (right panel) post induction.

The cells were fixed with 100% MeOH (5 min) and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab68194** at 0.5 µg/mL and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin, at 1/1000 dilution. Cells were then incubated with **ab150081**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown. Gamma is adjusted to 1.5 in all channels.

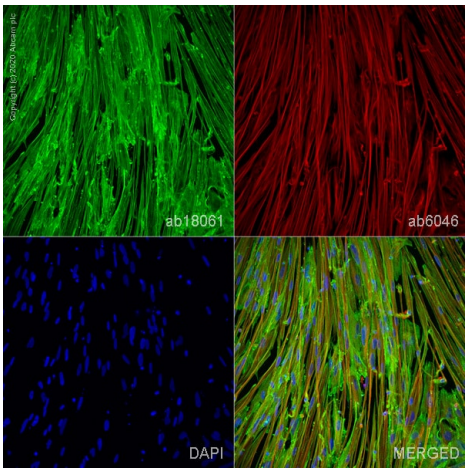


Immunocytochemistry/ Immunofluorescence - ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612)

Immunofluorescence staining of Actinin/ACTN1 using **ab68194** in ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612), which were differentiated for 3 (left panel), 5 (middle panel) and 10 days (right panel) post induction.

The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% PBS-Tween for 5 mins and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab68194** at 0.5 µg/mL and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin, at 1/1000 dilution. Cells were then incubated with **ab150081**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown. Gamma is adjusted to 1.5 in all channels.

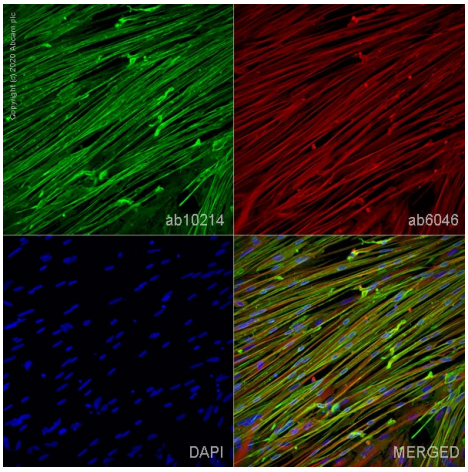


Immunocytochemistry - ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612)

Immunofluorescence staining of Actinin/ACTN1 using **ab18061** in ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612), which were differentiated for 10 days post induction.

The cells were fixed with 100% MeOH (5 min) and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab18061** at 5 µg/mL and **ab6046**, rabbit polyclonal to beta Tubulin, at 1/1000 dilution. Cells were then incubated with **ab150117**, Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150088**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown. Gamma is adjusted to 1.5 in all channels.



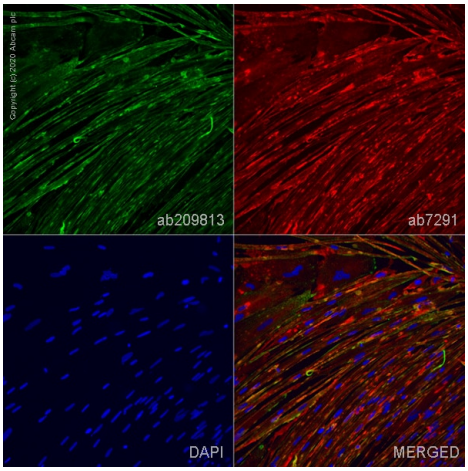
Immunocytochemistry - ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612)

Immunofluorescence staining of Cardiac Troponin T using **ab10214** in ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612), which were differentiated for 10 days post induction.

The cells were fixed with 100% MeOH (5 min) and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab10214** at 1 µg/mL and **ab6046**, rabbit polyclonal to beta Tubulin, at 1/1000 dilution. Cells were then incubated with **ab150117**, Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150088**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown. Gamma is adjusted to 1.5 in all channels.

The antibody **ab10214** also gave a positive staining using 4% formaldehyde fixation (10 min).



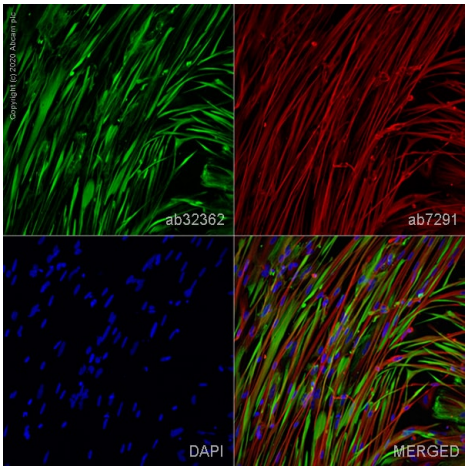
Immunocytochemistry/ Immunofluorescence - ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612)

Immunofluorescence staining of Cardiac Troponin T using **ab209813** in ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612), which were differentiated for 10 days post induction.

The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% PBS-Tween for 5 mins and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab209813** at 0.02 µg/mL and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin, at 1/1000 dilution. Cells were then incubated with **ab150081**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown. Gamma is adjusted to 1.5 in all channels.

The antibody **ab209813** also gave a positive staining using MeOH fixation (100%, 5 min).



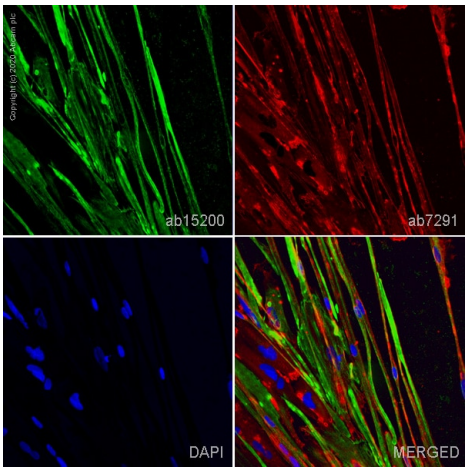
Immunocytochemistry/ Immunofluorescence - ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612)

Immunofluorescence staining of Desmin using **ab32362** in ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612), which were differentiated for 10 days post induction.

The cells were fixed with 100% MeOH (5 min) and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab32362** at 0.02 µg/mL and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin, at 1/1000 dilution. Cells were then incubated with **ab150081**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown. Gamma is adjusted to 1.5 in all channels.

The antibody **ab32362** gave comparable results using 4% formaldehyde fixation (10 min).



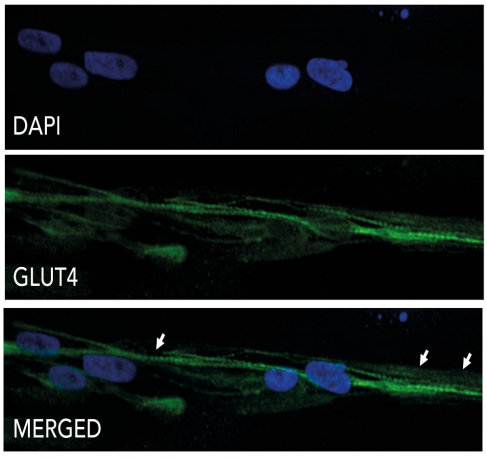
Immunocytochemistry - ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612)

Immunofluorescence staining of Desmin using **ab15200** in ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612), which were differentiated for 10 days post induction.

The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% PBS-Tween for 5 mins and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab15200** at 0.1 µg/mL and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin, at 1/1000 dilution. Cells were then incubated with **ab150081**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown. Gamma is adjusted to 1.5 in all channels.

The antibody **ab15200** gave comparable results using MeOH fixation (100%, 5 min).

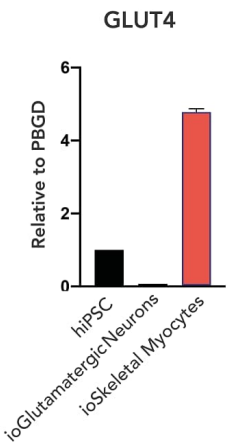


Immunocytochemistry - ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612)

Critically for metabolic studies, data demonstrates expression of the insulin regulated glucose transporter GLUT4 (Part 2)

Immunocytochemistry at Day 7 post-revival demonstrates expression of GLUT4 in peri-nuclear regions, and striations, in the ioSkeletal Myocytes.

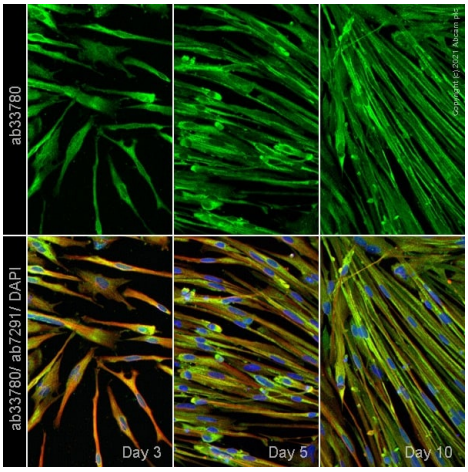
Image courtesy of Dougall Norris & Daniel Fazakerley, Wellcome-MRC Institute of Metabolic Science.



RT-PCR - ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612)

Critically for metabolic studies, data demonstrates expression of the insulin regulated glucose transporter GLUT4 (Part 1)

RT-qPCR at Day 10 post-revival demonstrates expression of GLUT4 in the ioSkeletal Myocytes, compared to undifferentiated hiPSCs and ioGlutamatergic Neurons.

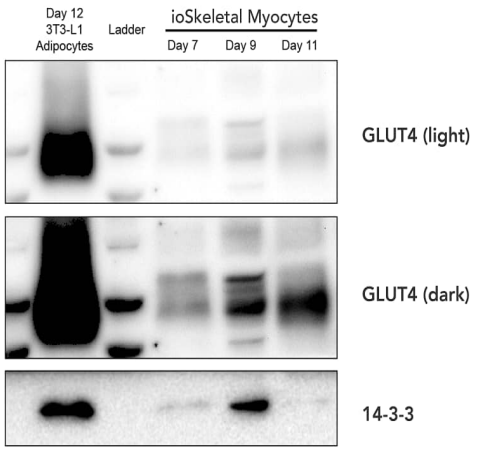


Immunocytochemistry/ Immunofluorescence - ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612)

Immunofluorescence staining of GLUT4 using **ab33780** in ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612), which were differentiated for 3 (left panel), 5 (middle panel) and 10 days (right panel) post induction.

The cells were fixed with 100% MeOH (5 min) and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab33780** at 5 µg/mL and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin, at 1/1000 dilution. Cells were then incubated with **ab150081**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown. Gamma is adjusted to 1.5 in all channels.

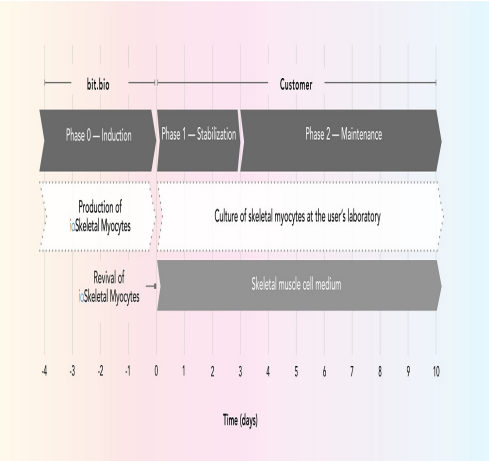


Western blot - ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612)

Critically for metabolic studies, data demonstrates expression of the insulin regulated glucose transporter GLUT4 (Part 3)

Western blotting of differentiated 3T3-L1 adipocytes and maturing ioSkeletal Myocytes demonstrates GLUT4 expression in a time-dependent manner.

Image courtesy of Dougall Norris & Daniel Fazakerley, Wellcome-MRC Institute of Metabolic Science.



Cell Culture - ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (ab277612)

ioSkeletal Myocytes cells arrive ready to plate. A simple one-medium protocol generates fully differentiated and mature skeletal myocytes.

The 3 phase protocol for generating ioSkeletal Myocytes:

1. Induction (carried out at bit.bio)
2. Stabilization for 3 days with Doxycycline
3. Maintenance during which the skeletal myocytes mature.

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours

- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors