Celluminova AB, c/o OBOE IPR
Box 1891, 581 18 Linköping
www.celluminova.se
info@celluminova.se

GlioStem®: Instructions For Use

1. Product description

Certain polymers, i.e., poly- and oligothiophenes, have been shown to be able to cross cell membranes without additional reagents and to illuminate when interacting with certain structures. GlioStem specifically detects neural stem cells and stem cell-like cells derived from glioma tumors. Within a maximum of 10 minutes after administration of GlioStem in vitro, in the existing media, fluorescence emission is observed without any modulation of the cells or additional reagents. Detection is efficiently assayed by fluorescent microscopy or fluorescence-assisted cell sorting (FACS). GlioStem has been tested in a large number of different cell types, and the specificity of GlioStem to detect neural stem cells and glioma stem cells in vitro has been verified in cell lines and primary cells from rodents as well as tumor tissue from humans. No secondary detection method (e.g., secondary antibodies or enzymatic reaction) or invasive technique is required.

1.1 Reactivity

GlioStem exposure results in a cytoplasmic luminescent signal clearly detectable at Alexa488/GFP wavelengths in specific cell types. GlioStem has been tested to positively identify embryonic cortical stem cells from rat (FGF2-expanded), embryonic stem cell-derived neural stem cells from mouse (FGF2/EGF-expanded), FGF2-exposed C6 glioma cell cultures from rat, hNES (human iPSC-derived neuroepithelial stem cells), progenitor-enriched cultures from human glioblastoma tumors and live tissue from human gliomas. In progenitor enriched cultures from human glioblastoma, GlioStem overlaps to >90% with CD271 (p75) staining in FACS experiments.

1.2 Reagent

GlioStem is supplied as 300 µg lyophilized powder to be reconstituted in 300 µl laboratory grade II water. The product is shipped at ambient temperature (15 – 25°C). Upon receipt, store immediately at the temperature recommended below. Avoid exposure to light.

1.3 Storage and stability

	Size	Storage	Shelf Life
Lyophilized GlioStem	300 µg	Store at 2 – 8°C	Stable until expiry date on label
Reconstituted GlioStem	300 µl (1 µg/µl)	Store at 2 – 8°C	Stable for up to 7 days after reconstitution

2. Directions for use

Please read the entire protocol before proceeding. This product should only be handled by users with laboratory experience and with knowledge in fluorescence microscopy and/or FACS.

2.1 Preparation

The lyophilized product is reconstituted in 300 µl laboratory grade II water for stock solution.

1. Pierce the rubber seal with a syringe and needle containing 300 µl laboratory grade II water.
2. Mix well by vortex and let stand for 1 minute at room temperature (15 – 25°C). Make sure that the powder is completely dissolved.
3. Dilute in preferred culture medium or PBS. Alternatively, add directly to existing medium prior to use. Do not save or reuse the working solution.

	Sample	Dilution
Microscopy	Cells	1:500
	Tissue	1:200
FACS	Cells	1:500
	Tissue	1:500

To obtain the best results, we recommend determining the optimal working dilutions by titration.

2.2 Microscopy of cultured cells

1. Discard culture medium and rinse cells with sterile PBS.
2. Add medium to cells and then add GlioStem directly into medium. Alternatively, add prepared medium with GlioStem already added.
3. Swirl plate and incubate for approximately 5 minutes at room temperature.
4. Detect in a fluorescent microscope at Alexa488/GFP wavelengths.

2.3 Microscopy of tissue

1. Prepare GlioStem in working solution in vial according to the table above. Medium or PBS can be used.
2. Put small tissue biopsies directly in the GlioStem-solution.
3. Incubate for 5 minutes at room temperature. Flick vial gently every 30 seconds for optimal staining in tissue.
4. Put biopsies on a microscope slide. Add a coverslip. No mounting media is necessary.
5. Detect in a fluorescent microscope at Alexa488/GFP wavelengths. The staining should show a high signal-to-noise ratio and be predominantly cytoplasmic.

Celluminova AB, c/o OBOE IPR
Box 1891, 581 18 Linköping
www.celluminova.se
info@celluminova.se

2.4 FACS of cultured cells

1. Discard culture medium and rinse cells with sterile PBS.
2. Add medium to cells and then add GlioStem directly into medium. Alternatively, add prepared medium with GlioStem already added.
3. Swirl plate and incubate for approximately 5 minutes at room temperature.
4. Safely detach cells from plate using a non-disruptive method that keeps the cell surface intact.
5. Analyze the cells on a flow cytometer immediately at Alexa488/GFP wavelengths.

2.5 FACS of tissue

All steps done at room temperature until staining with FACS antibodies.

1. Mince tissue using a no.10 scalpel in collection medium.
2. Add 5 ml of appropriate enzyme cocktail (depending on tissue).
3. Incubate with rotation for 60 minutes at 37°C.
4. Pass tissue-enzyme mix through a 70 µm nylon strainer.
5. Centrifuge at 350 g for 7 minutes.
6. Wash twice with HBSS (to remove all enzymes).
7. Lyse red blood cells (if necessary) with 1X red cell lysis buffer.
8. Leave for 10 minutes at room temperature.
9. Centrifuge at 350 g for 7 minutes. In the meantime, prepare percoll gradients (if necessary, to remove myelin or fat cells).
10. Resuspend cells in 500 µl 1x HBSS.
11. Slowly overlay cells resuspended in HBSS over percoll gradients in a 15 ml tube.
12. Spin cells using appropriate time and force depending on tissue and percoll gradients. Make sure to change acceleration to 4 and the break to 0 on the centrifuge.
13. Remove cells from appropriate layer and add 10 ml HBSS to wash the remaining percoll from the cells.
14. Spin at 350 g for 7 mins.
15. Resuspend unstained control in FACS buffer (PBS containing 2.5% BSA and 5 µM EDTA) and the remaining sample in 500 µl of staining mix containing antibodies of interest.
16. Incubate cells for 15 minutes on ice.
17. Add 5 ml of FACS buffer and spin at 350 g for 5 minutes.
18. Go immediately to the flow cytometer. Add DAPI at appropriate concentration to assess cell death.
19. Leave sample at room temperature for approximately 10 minutes.
20. Add GlioStem (1:500) for 5 minutes.
21. Analyze the cells on a flow cytometer.
22. Detect GlioStem using Alexa 488/GFP/FITC filter.

3. Recommendations

- GlioStem only works in live cells or tissue that are viable and have intact cell membranes.
- Identification of GlioStem in heterogeneous tumor tissue samples can be improved by using a myelin removal kit and percoll for removal of red blood cells.
- Change to medium without phenol red prior to FACS-sorting of cells in order to eliminate background signals.
- Do not wait longer than 10 minutes to start microscopy or FACS of GlioStem-stained sample. Background levels may increase with longer exposure.
- Do not use high exposure times in the microscope as damaged cell-membranes will lead to increased background.
- Optimal incubation times may vary between cell types.
- GlioStem can be used together with DAPI for microscopy.
- GlioStem can be used together with other surface-markers for FACS.
- Product shall be discarded according to local regulation for chemical waste (unused product) and biological waste (used product).
- Product is not for resale.
- Contact Celluminova for Material Safety Data Sheet regarding information and safe handling practices.

 Manufacturer  Date of manufacture  Use by date  Batch code  Catalogue number  Non-sterile

 Do not use if package is damaged  Keep away from sunlight  Temperature limit  Consult instructions for use

 In vitro diagnostic medical device  CE certified