Antibleaching live cell visualization medium DMEM^{gfp}-2

- Recommended for visualization of cells expressing green fluorescent proteins

Photobleaching of fluorescent proteins in response to prolonged exposure to exciting radiation significantly impacts their performance as *in vivo* labels. It has been shown that oxidative reddening of green fluorescent proteins is one of the main sources of GFP photobleaching [1]. Evrogen's new DMEM^{gfp}-2 is a minimally depleted live cell visualization medium that excludes the components responsible for this effect [2]. DMEM^{gfp}-2 is an improvement over DMEM^{gfp} (referred to as DMEM-V in [1]), exhibiting similar green fluorescent protein photostability without depleting as many of the compounds normally present in most common cell culture media. This modification makes DMEM^{gfp}-2 better suitable for long-term experiments.

Replacing the culture medium with DMEM^{gfp}-2 for the period of visualization results in up to a 9-fold increase of photostability of EGFP, a 3.3-fold increase of photostability of TagGFP2 and more than a 4-fold increase of photostability of activated forms of photoactivatable PA-GFP and PS-CFP2 (the effect varies for particular fusions and localizations).

Rutin (plant flavonoid glycoside also known as vitamin P) is the component that inhibits oxidative reddening and thus increases EGFP photostability in the cell culture experiments [2]. An increase of EGFP photostability comparable to the level observed in DMEM^{gfp}-2 is achieved when rutin is added 30 min before imaging to standard DMEM. Addition of rutin to DMEM^{gfp}-2 results in further enhanced EGFP photostability (approximately 1.5-fold).

Product	Cat.#	Description	Size
DMEM^{gfp}-2	MC102	antibleaching live cell visualization medium	100ml
DMEM^{gfp}-2 kit	MCK02	DMEM^{gfp}-2 antibleaching live cell visualization medium; Rutin, 100x solution	100ml 1ml

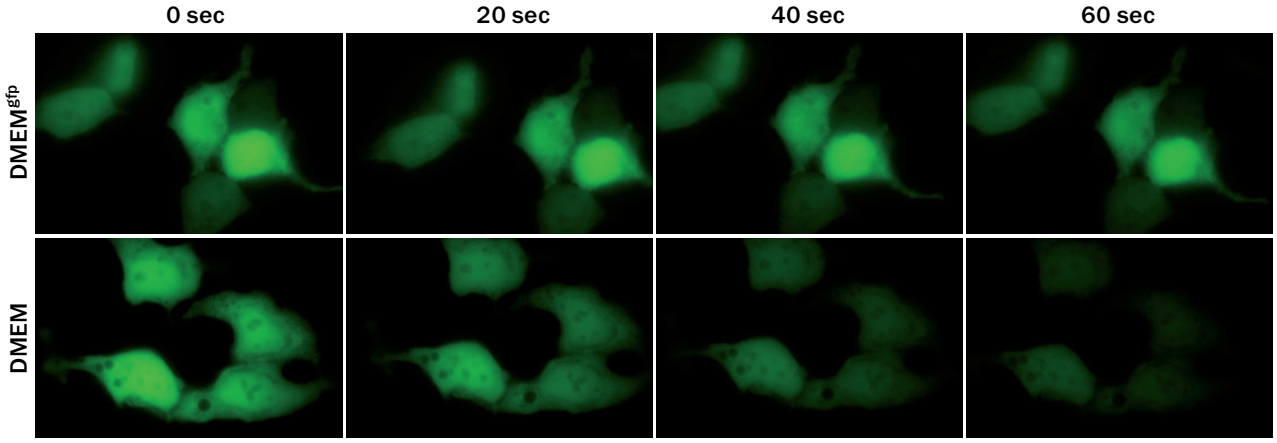
Shipping: ambient temperature.

Storage: DMEM^{gfp}-2 medium should be stored at +4 – +8°C. Rutin is stored at -20°C.
Upon receipt place rutin in the freezer. DO NOT FREEZE DMEM^{gfp}-2.

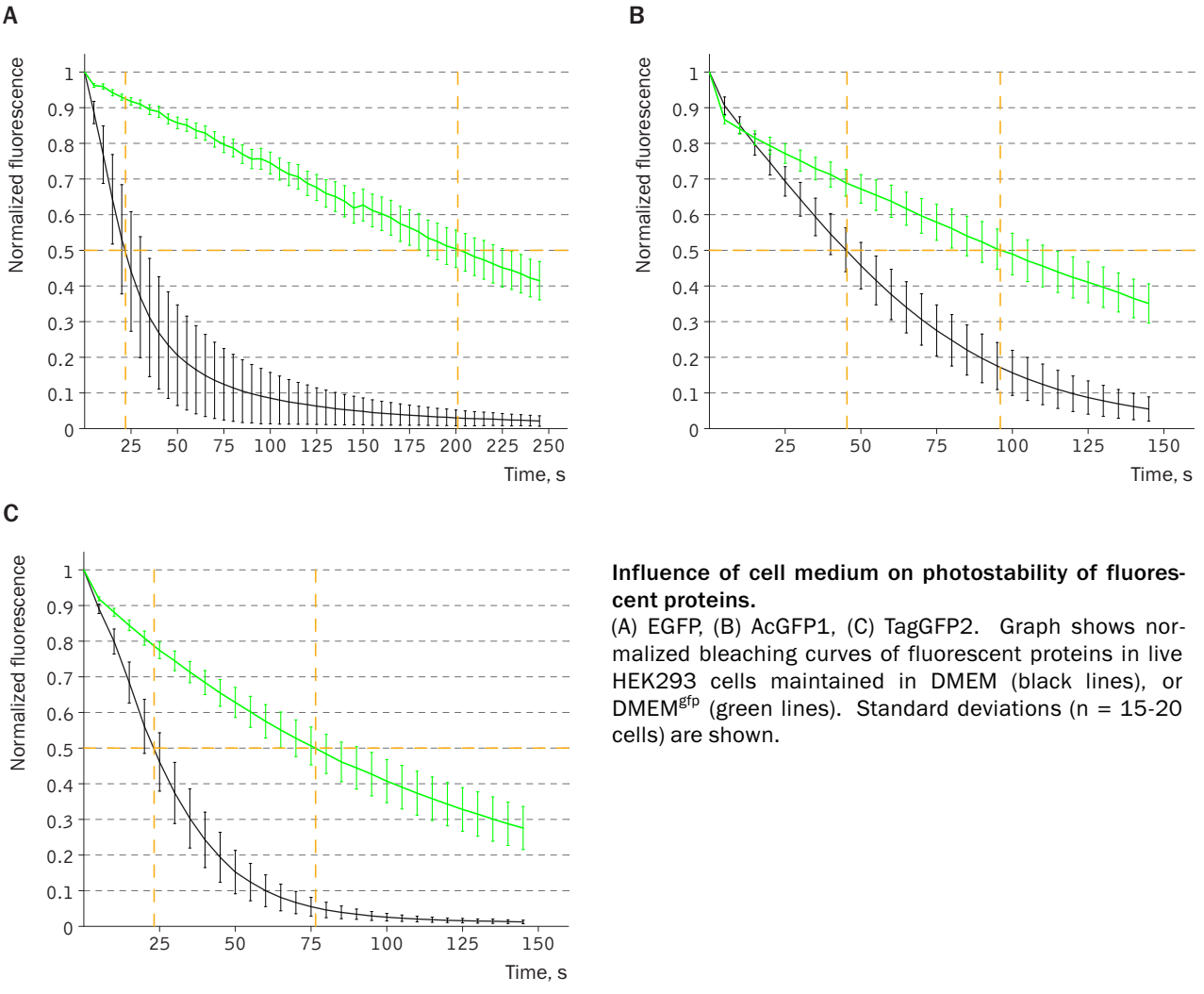
Use

30 min before starting imaging experiment replace the cell culture medium with DMEM^{gfp}-2 as is or supplemented with rutin at a final concentration of 20 mg/l. Always prepare a fresh solution of rutin in DMEM^{gfp}-2! The replacement should be performed under sterile conditions.

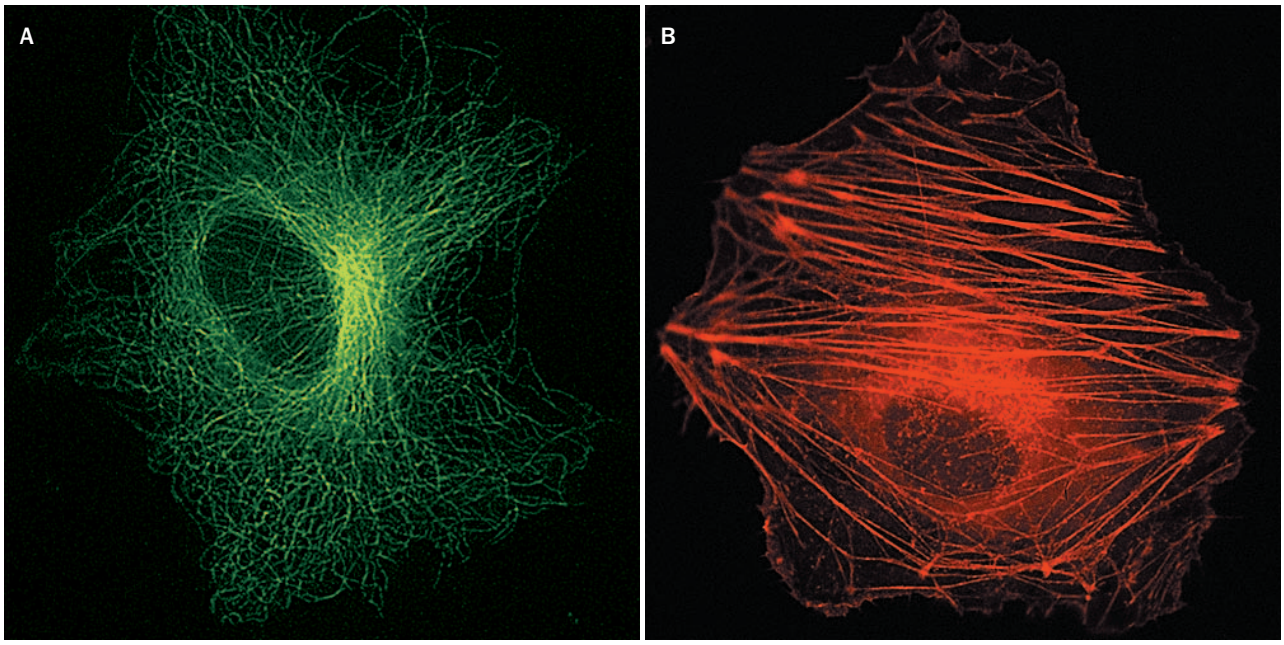
For long experiments DMEM^{gfp}-2 can be supplemented with L-glutamine, penicillin, streptomycin and fetal bovine serum.



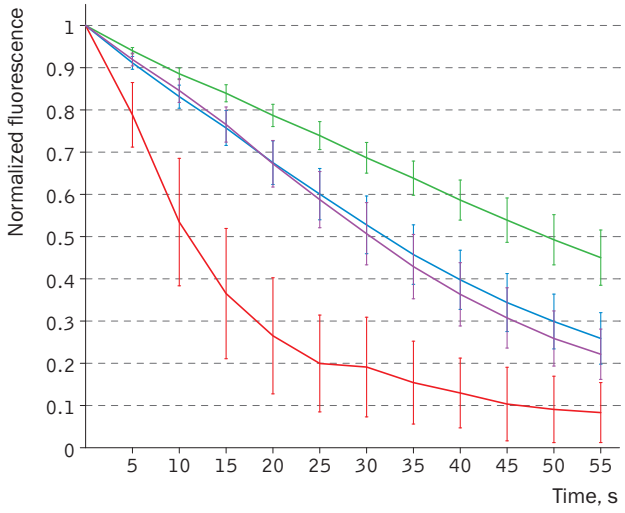
Long-term fluorescent microscopy of EGFP in live HEK293T cells maintained in DMEM or DMEM^{gfp}.



Influence of cell medium on photostability of fluorescent proteins.
(A) EGFP, (B) AcGFP1, (C) TagGFP2. Graph shows normalized bleaching curves of fluorescent proteins in live HEK293 cells maintained in DMEM (black lines), or DMEM^{gfp} (green lines). Standard deviations (n = 15-20 cells) are shown.



HeLa cells transfected with fluorescent protein-tagged α-tubulin or β-actin had a normal cytoskeleton after 5-day culture in DMEM^{gfp}.
Fluorescence microscopy of TagGFP2-α-tubulin(A) and mKate2-β-actin(B) of live HeLa cells after 5 day culturing in DMEM^{gfp}.



Influence of rutin on EGFP photobehavior.
Bleaching of green fluorescence in EGFP-expressing live HEK293 cells maintained in DMEM (red), DMEM with rutin (blue), DMEM^{gfp}-2 (violet), or DMEM^{gfp}-2 with rutin (green). Green fluorescence intensities in individual cells are background subtracted and normalized to maximum (initial) value in each cell. Standard deviation values (n = 15–20 cells in a representative experiment out of five independent experiments) are shown.
Data from [2].

References

[1] AM Bogdanov et al. (2009). Nat Methods. 6 (12): 859–860 / pmid: 19935837
[2] AM Bogdanov, EI Kudryavtseva, and KA Lukyanov. (2012). PLoS One. 7 (12): e53004 / pmid: 23285248

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