Chemical Labelling Flies

Jonny Kohl, Julian Ng and Greg Jefferis

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Thank you for your in interest in the chemical labelling flies that we have developed. Please see [Kohl et al., 2014] for protocols and all the experimental details you should need to use these flies.

1 General Staining

In order to carry out the most basic test we recommend (in order):

1. X-Gal4 x UAS-myr-SNAP

or 2. X-Gal4 x UAS-myr-Halo

These both work very well in our hands but there is a wider choice of SNAP ligands. The best reagents to start with are the red dyes.

Tag	Dye	Catalogue
SNAP	SNAP-Surface 549	S9112S
SNAP	SNAP-Surface 488	S9124S
Halo	HaloTag TMR Ligand	G8252

We have been using the ligands at 1µM. At this concentration ligands are priced at about £1-£2 per 500µl staining.

2 Neuroanatomy

The most interesting line is probably:

brp-SNAP; UAS-myr-Halo

You can use these reagents:

Tag	Dye	Catalogue
SNAP	SNAP-Surface 488	S9124S
Halo	HaloTag TMR Ligand	G8252

3 Notes

Please read the "Protocol for chemical labeling of Drosophila brains" in the supplemental information.

3.1 Signal

Remember that SNAP etc staining is quantitative (i.e. one fluorophore binds per tagged protein) whereas antibodies will often boost signals at the weaker end of the dynamic range (as well as giving more background). We have been very pleased with the results with our regular Gal4 lines, where we generally find superior SNR with these reagents, but it is likely that both signal and background are lower compared with primary+secondary antibodies. We are working on flies with multiple copies, multimerised transgenes etc.

3.2 Time

Most of our tests were done with 15min incubations, but we have seen that incubations of 5 min give good results with 1µm concentration ligands and we know that incubation times as low as 1 min can still give good signal with brp-SNAP knockin (see Fig S5d).

3.3 Concentration

We have some preliminary tests that suggest that ligand concentrations up to 10x lower (i.e. 100nM) can also work (see Fig S5e), but may need longer staining times for full staining (15-30min?). We would welcome feedback on labelling time/concentrations that work for you.

4 Terms and Conditions

If we are sending these flies to you ahead of publication (acceptance), you are free (and encouraged!) to test them extensively, subject only to the following

1. We want your feedback! Please send us a picture with a test staining!
2. In particular, if you have trouble, please contact us at tissuetagging@googlegroups.com.
3. If someone outside your lab wants the flies before publication, please tell them to contact Greg at jefferis@mrc-lmb.cam.ac.uk
4. Please don't publish any experiments using these reagents without checking the state of our manuscript – we hope it will be fast!
5. If you develop a major new project based specifically on these reagents before our manuscript is published, then please get in touch with Greg to discuss – for one thing, we might have developed some useful new reagents.
6. Good luck! And tell your friends – especially if you're happy with the results!

5 Support and Feedback

Please send any technical queries to tissuetagging@googlegroups.com – that way you'll get a faster response and others can benefit from the information. To share feedback please write to the group unless you have e.g. test image data that you want to share in private. For this and any non-scientific queries then write to Greg at jefferis@mrc-lmb.cam.ac.uk. Thanks again!

References

Johannes Kohl, Julian Ng, Sebastian Cachero, Michael-John Dolan, Ben Sutcliffe, Daniel Krüger, Shahar Frechter, and Gregory SXE Jefferis. Ultra fast tissue staining with chemical tags. *bioRxiv*, 2014. doi: 10.1101/005298. URL http://biorxiv.org/content/early/2014/05/19/005298.