

<Preparation of TSA-Biotin>

1. Dissolve 15 mg of Tyramine hydrochloride 15 mg into 300 μ L DMSO.
*Tyramine hydrochloride: SIGMA #T2879-1G (MW=173.65)
2. Dissolve 3.5 mg Biotin-NHS into 36.5 μ L DMSO.
* Biotin-NHS: CALBLOCHEM #203112 (MW=341)
3. Mix 36.5 μ L each of Tyramine solution and Biotin-NHS and incubate at room temperature overnight under dark condition.
4. Aliquot the reaction mix and stock frozen.

<Note>

- > The reaction may be terminated by addition of 1/10 volume of monoethanolamine.
- > The above recipe is for a test scale. We recommend scaling up the reaction and use all the Biotin-NHS powder left. We can't keep the powder at 4°C for a long time.
- > This recipe originally came from Mike Dyer's protocol (Cepco lab)
<http://genepath.med.harvard.edu/~cepko/protocol/mike/T4.html>
- > The application of this protocol for enhancing immunostaining can be found in Kaneko lab's paper.

Kuramoto E, Furuta T, Nakamura KC, Unzai T, Hioki H, Kaneko T.

Two types of thalamocortical projections from the motor thalamic nuclei of the rat: a single neuron-tracing study using viral vectors. *Cereb Cortex*. 2009 Sep;19(9):2065-77. PMID: 19174446