**In-gel fluorescence staining of FlAsH-tagged recombinant proteins from TX-TL**

Prepare the following:

**4x SDS-PAGE loading buffer**

200 mM Tris-HCl (pH 6.8)

8% (w/v) sodium dodecyl sulfate

0.4% (w/v) bromophenol blue

40% (v/v) glycerol

**FlAsH-EDT2 dilution**

30 mM FlAsH-EDT2 in 50 μL of DMSO

Dilute to 1 mM (10x) in ddH2O – slightly insoluble, vortex before pipetting in next steps

**0.5 M TCEP—5 mL**

**Sample preparation**

1. For a 33 μL cell-free sample, add 1 mL of ice-cold acetone.
2. Store in freezer for 1 h, or freeze on dry ice.
3. Spin down at 16,000 × *g*, 10 min, RT.
4. Remove supernatant.
5. Wash pellet with 1 mL of ice-cold 70% acetone
6. Spin down at 16,000 × *g*, 10 min, RT.
7. Remove supernatant and dry pellet.
8. Add the following:
9. 22 μL ddH2O
10. 10 μL of 4x SDS-PAGE loading buffer
11. 4 μL of 0.5 M TCEP
12. Boil for 5 min.
13. Add 4 μL of 1 mM FlAsH-EDT.
14. Incubate for 15–30 min at RT.
15. Centrifuge for 10 min, 16,000 × *g.*
16. Add 5 μL of supernatant onto SDS-PAGE following the manufacturer’s guidelines. Avoid overloading, as this leads to smearing of the bands. Optional: run a fluorescent protein ladder (Biorad - Precision Plus Protein™ WesternC™ Blotting Standards) or purified FlAsH-tagged standard protein in parallel.
17. Visualize unstained gels on an ultraviolet light-box or by using a camera and Xe-light source with appropriate filters for FlAsH fluorescence (excitation at 480 nm, with emission at 535 nm).
18. Optional: Gels can be stained with routine Coomasie Blue staining or used for western blotting analysis.