Dot blot analysis of in vitro RNA labeling

1. 200 ng μl−1 RNA was incubated with 100 μM photosensitizer (miniSOG) and 5 mM amine-containing probe (biotin-conjugated probes or alkyne-conjugated probes) in a 50 μl reaction in 200 μl PCR tube;
2. Illumination with 10 W blue LED for 30 min at room temperature
3. RNA was purified from the reaction mixture with RNA Clean & Concentrator™-25 (ZYMO, **Catalog #**R1017, R1018): Add 2 volumes RNA Binding Buffer to each sample1 and mix. Add an equal volume of ethanol (95-100%) and mix. Transfer the sample to the Zymo-Spin™ IICR Column3 in a Collection Tube and centrifuge. Discard the flow-through. Add 400 μl RNA Prep Buffer to the column and centrifuge. Discard the flow-through. Add 700 μl RNA Wash Buffer to the column and centrifuge. Discard the flow-through. Add 500 μl RNA Wash Buffer to the column and centrifuge for 2 minutes ensure complete removal of the wash buffer. Carefully, transfer the column into a RNase-free tube. Add 30-50 μl DNase/RNase-Free Water directly to the column matrix and centrifuge.
4. Equal volume of purified biotinylated RNA was loaded onto PVDF membrane and crosslinked to the membrane by an ultraviolet light
5. The membrane was blocked in 5% BSA at room temperature for 1 h and incubated with Streptavidin-HRP at room temperature for 1 h
6. The membrane was washed three times with TBST (50 mM Tris, 150 mM NaCl, 0.1% Tween-20, pH 7.4–7.6) for 10 min each time, incubated in Clarity Western ECL Substrate (Bio-Rad, 1705061) and then imaged on a ChemiDoc imaging system