

In vivo protocol

1. Inject 40 nmol puromycin / g body weight i.v. and 10 min later harvest tissue for Western blot analysis. Also harvest tissue from an animal not treated with puromycin, and analyze in parallel.
2. Homogenize the tissue, centrifuge 2,000 x g for 3 min at 4°C, and load 20 µg of protein onto a SDS-polyacrylamide gel.
3. Transfer the protein from the gel to a PVDF membrane, block the membrane, and then incubate overnight at 4°C with anti-puromycin antibody at a concentration of 1 µg/ml in Tris-buffered saline containing Tween-20 (TBS-T).
4. Wash the membrane in TBS-T, and incubate in goat anti-mouse HRP secondary antibody for 1 hr (Bethyl Laboratories, 1:10,000).
5. Develop using ECL reagent.

Cells in culture protocol

1. Incubate cells with puromycin (1 µM final concentration) for 30 min.
2. Harvest the cells, centrifuge 1,000 x g for 3 min at 4°C, and load 15 µg of protein onto a SDS-polyacrylamide gel.
3. Transfer the protein from the gel to a PVDF membrane, block the membrane, and then incubate overnight at 4°C with anti-puromycin antibody at a concentration of 1 µg/ml in Tris-buffered saline containing Tween-20 (TBS-T).
4. Wash the membrane in TBS-T, and incubate in goat anti-mouse HRP secondary antibody for 1 hr (Bethyl Laboratories, 1:10,000).
5. Develop using ECL reagent.