Puromycin ELISA protocol

1. Dispense 100µl Coating Buffer per well in the required number of wells in a 96 well ELISA plate. Dilute and mix the Protein extract (in the same buffer the sample is in) to 300ng/µl. Immediately dispense 1µl /well into plate. Do not store the diluted sample and re-use.
2. Incubate the plate at 37C (without CO_2) for 2 hrs.
3. Vigorously shake out contents. Wash 1x with 200ul PBS.
4. Add 200µl Blocking Solution. Incubate 30 min at RT.
5. Dilute Puromycin antibody in Blocking solution at 100ng/ml.
6. Remove Blocking Soln. by vigorously shaking. Add 100µl Antibody (diluted in Blocking Solution) per well.
7. Incubate 1hr at RT.
8. Wash 2X with 200ul PBS
9. Add 100µl Secondary Antibody in Blocking Soln. Incubate 1 hr at RT.
10. Wash 4X 200µl PBS. Shake out contents vigorously.
11. Add 100µl Substrate. Stop with 100µl of Stop solution when the blue develops enough (typically 5-20 min).

Maximal binding control

Prepare 1-2 dilutions of one of the "highest signal Puromycin containing samples" in coating buffer and plate 1200ng, 600ng, 300ng, 150ng, 75ng, and 37.5ng per well.

Materials

- **U Bottom Micro Titer plates VWR# 62402-954**
- **Coating Buffer**
  - 50mM Sodium Bicarbonate pH 9.6 (store 4C), MW 84.01
- **PBS**
- **Blocking Solution**
  - 5% BSA in PBS
- **Puromycin Monoclonal Antibody**
- **Peroxidase Secondary Antibody Dilution**
  - Jackson Goat anti-mouse 415-035-166, 1:1000
  - Rehydrate with water as indicated on spec sheet. Add an equal volume of Glycerol. It already has BSA in it. Mix gently. Store at -20C.
- **Biofx TMB Super Sensitive HRP Substrate** #TMBS-0100-01 (Surmodics.com)
- **Biofx 450nm Stop Buffer** #STPR-0100-0 (Surmodics.com)
- **Plate reader**