Biotin Rapid Assay Protocol
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Date:_____
Scientist:_____

Description: This kit is used to quickly measure biotin level in liquid samples (<2 hours) in a range of 0.3125ng/ml to 20ng/ml with a competition enzyme assay. The kit includes one 96-well plate suitable for measuring 40 samples in duplicates; biotin standard, substrate and stopping solution. If original sample is in solid form, use either TBST or water to extract biotin since it is water soluble.
Note that milk product and BSA contains biotin and thus are not suitable to use in the assay.

Materials:

Provided in the kit:
One 96-well plate coated with avidin
Biotin (4ug/ml, 50ul)
Biotinylated-HRP (50ul, use at 1:1000 dilution)
Substrate (8ml)

Customer prepare:
TBST (TBS + 0.05% Tween 20, see below)
1.5ml or 2ml tubes
HCl, 1M, 5ml
TBST:

NaCl	8g
KCl	0.2g
Trizma Base	1.21g
D.I. Water	1L

Adjust pH to 8.6 with 1M HCl
0.5ml Tween-20 (Polyoxyethylene-Sorbitan Monolaurate

Procedures:

1. Prepare tested sample either in TBST, PBS or water if sample is not in liquid form

2. Dilute 20ul of Biotinylated-HRP in 20ml TBST

3. Make biotin standards: prepare 7 tubes labeled with 20ng/ml, 10ng/ml, 5ng/ml, 2.5ng/ml, 1.25ng/ml, 0.625ng/ml and 0.3125ng/ml; add 1ml above diluted Biotinylated-HRP to tube 20ng/ml and 0.5ml/tube to the other six tubes; add 5ul of the provided 4ug/ml Biotin to tube 20ng/ml and mix well and take out 0.5ml into the 10ng/ml tube and mix well, continue the serial 2X dilution until the last tube (0.3125ng/ml)

4. Remove plastic cover from the plate and wash the wells twice with TBST

5. Wells A1-A2 to H1-H2 can be used for establishing standard curve: add 100ul/well of diluted Biotinylated-HRP from step 3 to wells: A1-A2 (20ng/ml), B1-B2 (10ng/ml), C1-C2 (5ng/ml), D1-D2 (2.5ng/ml), E1-E2 (1.25ng/ml), F1-F2 (0.625ng/ml), G1-G2 (0.3125ng/ml) and 100ul/well of diluted biotinylated-HRP (step 2) to H1-H2 (as zero biotin control)

6. Add sample: 1ul/well to 5ul/well in duplicate depending on the possible biotin content and then add 100ul/well of Biotinylated-HRP (step 2)

7. Mix the plate on an ELISA Plate shaker at 400rpm for 1 hour at RT.

8. Wash 5X with TBST.

9. Add 50ul/well substrate and let the blue color to develop 2-5 minutes or until blue color like blue tip appears in H1-H2.

10. Stop the reaction with 50ul 1M HCl. and read OD450 immediately.

11. Record the development time on the ELISA recording sheet along with OD450 readout.

12. Establish standard curve according to readouts (duplicate averages) from A1-A2 to H1-H2 and estimate the sample biotin concentrations based on the standard curve.

Following is an example of assay standard curve