



**Start**  
 50 µl Patient Samples  
 Standards  
 Control serums

200 µl Extraction Buffer +  
 Biotin

Shake for  
 30 Seconds

22-28° Incubation  
 In room temp.  
 No light  
 60 minutes

300 µl Washing solution  
 Soak for 10 Seconds  
 Every time

**boors teb**  
 بازار تجهیزات پزشکی ایران  
 25-OH-Vitamin D  
 Assay procedure flowchart  
 Bti ( behshad tajhiz Esfahan )

100 µl Chromogen  
 Substrate A+B

300 µl Washing solution  
 Soak for 10 Seconds  
 Every time

22-28° Incubation  
 In room temp.  
 No light  
 30 minutes

100 µl HRP – Avidine  
 Conjuget

22-28° Incubation  
 In room temp.  
 No light  
 15 minutes

100 µl Stop solution

Reading in  
 450-630 nm

Calculation

STEP 3

STEP 3

