EVix High Sensitivity Tetraspan Tracking (HSTT) Kit

For research use only. Not for diagnostic or therapeutic procedures

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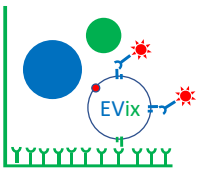


EVix High Sensitivity Tetraspan Tracking (HSTT) Kits quantify human extra-cellular vesicles (EV). CD9, CD63, and CD81 positive EVs are captured. Captured EVs are detected with a mix of anti CD9, CD63 and CD81 biotinylated antibodies.

Use to quantitate EV preparations by antigen content not particle counting. HSTT kits are used to quantitate EV preparations for use in Multi Tope and Single Tope assays.

Kit Contents: If contents are unopened and stored at 4° C the kit is stable for 6 months. A: EVix Capture Plate: (12 x 8 well strips) and frame. **B:** 10X Sample and Wash Buffer Concentrate: 2 bottles (10 ml each) to make 200 ml. **C:** Biotinylated Primary Detection Antibody: 1 vial of 0.5 ml to make 10 ml. **D:** Streptavidin Horse Radish Peroxidase SAHRP Conjugated: 1 vial of 0.5 ml to make 10 ml. **E:** Chromogenic Solutions : 1 bottle (10 ml) of stabilized H2O2 , 1 vial (0.5 ml) of stabilized TMB chromogen. **Caution! F:** Stop Solution: 1 bottle (10 ml) **Acid Caution! G:** EVTetraspan Standard: 1 vial 0.5 ml.

Incubation Time and Temperature: A 2 hour capture incubation at RT is recommended. This is followed by a 1 hour incubation with detection antibodies at RT. Shaking is necessary to assure maximum interaction between the EV and the capture antibody. If you alter these times, control and record them to reproduce your results.

EV Tetraspan Standard: EVix standard added to 1X wash buffer at a 4 fold dilution yields a positive signal near 3 to 4 AU. Adding 50 ul of standard to 150 ul of 1X wash buffer, yields a dilution when assayed at 100 ul per well, gives a strong positive signal. It can be further diluted stepwise to provide a reproducible standard line. The standard preparation is depleted of proteins below 300,000 MW. Each kit contains 0.5 ml of standard which is sufficient to run each 8 well strip independently if desired. Store at -70 C.

Assay Protocol: Depending upon the size of assay, select the number of wells. Strips of wells not being used can be removed from the frame and stored at 4° C in resealable foil pouch. All kit components are provided in volumes sufficient to run 96 wells. Dilute only what is needed for the assay and store the remaining concentrate at 4° C.

1. Capture EVs (2 hour): Each assay run should include a positive control (EVix Standard) and a negative control (Wash buffer only, no antigen) along with samples. To quantify samples, run several dilutions in duplicate. Samples are added directly to the assay well in 100 ul volumes. Use 1X wash buffer to dilute samples and standard as needed. After 2 hour capture, aspirate the capture volume. The well is ready for the addition of detection antibody.

2. Detection antibody (1 hour): Add 100 ul of diluted biotinylated detection antibody solution to each well in the assay. Detection antibody is diluted 21 fold for use. If the entire plate is used, add 0.5ml of detection antibody solution to 10 ml of 1X Wash Buffer. If fewer wells are used , dilute accordingly. For example if only 1 ml of detection antibody is needed, dilute 50 ul of concentrated antibody into 1.0 ml of 1X Wash Buffer. 1 hour incubation at room temperature with shaking has been shown to be optimal. After 1 hour, aspirate the well volume. Add 300 ul of wash buffer, and aspirate. The empty well is ready for the next step, addition of 100 ul Streptavidin Horse Radish Peroxidase (SAHRP). Once diluted, biotinylated detection antibody is stable 1 week at 4° C.

3. SAHRP (20 minute): Add 100 ul of diluted SAHRP solution to each empty well. SA HRP is diluted 21 fold before use. To make 10 ml of SA HRP add 0.5 ml (vial contents) of concentrate to 10 ml of 1X Wash Buffer. To make 1.0 of working SA HRP solution, add 50 ul of concentrate to 1.0 ml of 1X Wash Buffer. Plate should be shaken at room temp for 20 min.

4. Wash 3X: After SAHRP incubation, wells receive 3 washes of 300 ul. Well contents are aspirated and 300 ul Wash Buffer is added. This is done 3 times and final volume is aspirated prior to addition of chromogen.

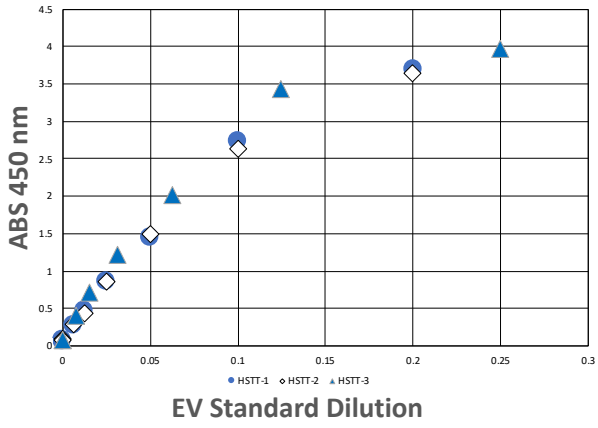
5. Chromogen Addition (10 minute): The chromogen solution is added in 100 ul volumes to each washed well. Prepare the chromogen solution just prior to use, so that it is fresh when added to the assay. It is not stable and

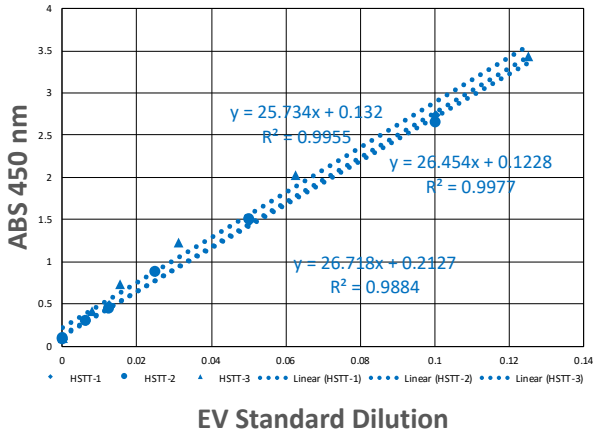
should be used within 10 minutes of mixing. The chromogen solution is prepared by adding 0.5 ml of part A to 10 ml of part B. To make 1.0 ml of solution add 50 ul of part A to 1.0 ml of part B. The enzymatic reaction is carried out for 10 min. The incubation time is important, to provide equivalent results from assay to assay. At 10 minutes add stop solution. **CAUTION: These components are considered hazardous and should be handled accordingly.**

6. Stop Solution: Stop the colorimetric reaction after 10 minutes. All wells receive 100 ul of stop solution added to the 100 ul chromogenic solution already in the well. Wells will change from blue to yellow upon addition of the stop solution. **CAUTION. Stop solution is 1N Sulfuric Acid and should be handled accordingly.**

7. Read Absorbance: Read the optical absorbance of each well at 450 nm wave length within 10 minutes after addition of the stop reagent.

Data Analysis: The EV standard is provided as a positive control. Dilution of the EVix standard is shown below.





EV quantitation and comparison Analysis of multiple EV preparations from different cell cultures and biological specimens is shown below. The values for EV preparations diluted as shown on the horizontal axis indicate that each preparation has its own EV concentration. Comparison of an unknown preparation to your known preparation can be done for comparison. Once your EV preparation has been characterized, the HSTT assay can be used to adjust other EV preparations to equivalent concentrations.