SPHERO™ Technical Note **STN-3 Rev B 110306**

DETERMINATION OF THE BINDING CAPACITY OF AVIDIN MAGNETIC PARTICLES

MATERIALS:

1. Avidin magnetic particles, 1% w/v, Cat. # VM-40-10, Lot No. J01, 4.35 µm

2. Biotin-FA conjugate, Lot No. 021688, 533 nM in 1% diluent. (IBS containing 1% normal goat serum and 1% fetal bovine serum)

PROCEDURES:

1. Add 50, 100,150, 200, 300 & 400 µl of Avidin magnetic particles to six 12x75 test tubes.

2. Add 1 mL of Biotin-FA conjugate to each tube.

3. Vortex and incubate at ambient temperature for at least 30 minutes with occasional shaking.

4. Adjust the fluorimeter for excitation and emission at 490 and 520 nm respectively.

5. Set 100% emission with the Biotin-FA conjugate.

6. Separate the magnetic particles and read the fluorescence of the supernatant for each tube. Normalize with the dilution factor in each tube.

7. Fluorescence reduction is proportional to the Biotin-FA bound to the Avidin magnetic particles.

RESULTS:

The binding capacity of Avidin magnetic particles is approximately 0.12 nmole of Biotin-FA per mg of particles as shown in Fig. 1.