

# SpheroTECHNICAL NOTES

STN-2 Rev. A 120293

---

## Determination of Antibody Binding to Particles:

The amount of the antibody binding to the particles after coating can be determined by using the subtraction method as follows:

1. Measure the absorbance of antibody solution to be used for coating at 280 nm (OD<sub>280</sub>). The IgG has OD<sub>280</sub> of ~1.4/mg.
2. Pellet the particles to be coated by centrifugation or magnetic separation.
3. Resuspend the particles in the antibody solution from step 1.
4. After coating the particles, aspirate and save the supernatant from the first centrifugation or magnetic separation.
5. Filter the supernatant through a 0.2 µm Acrodisc and measure the OD<sub>280</sub> of the filtrate which contains unbound antibody. Normalize the OD<sub>280</sub> reading to the same volume as in Step 1.
6. The % reduction in OD<sub>280</sub> reading from Step 5 as compared to Step 1 indicate the the % of antibody bound to the particles.

**Spherotech, Inc.**

1840 Industrial Dr., Suite 270, Libertyville, IL 60048