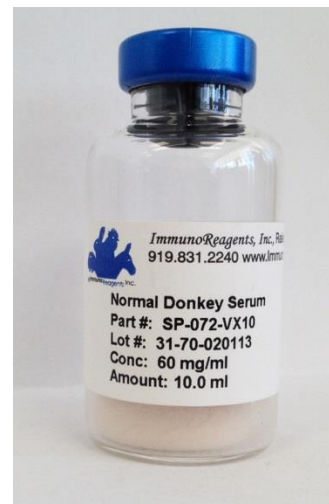


Serum Blocking Buffer

Blocking is a critical step in most immunoassays. Blocking “fills-in” the unoccupied spaces of the solid phase that are not occupied by immobilized proteins. Blocking prevents non-specific binding of the antibodies. Without blocking agents, the antibodies could bind without specificity and lead to false signaling and/or background issues. While there is no single “best” blocking agent, empirical testing is needed to validate which methods works best in each assay. **In general, a mixture of proteins combined with nonionic detergents is most often used.*



How to Choose Which Serum:

When choosing which normal serum to use, it is recommended to use serum that is derived from the same host species as the secondary antibody. For example:

Secondary Antibody = Donkey anti-Rabbit IgG-HRP

Blocking Serum = Donkey Serum

**CAUTION: Do not use serum from the same species as the Primary Antibody, as this will compete for sites with the secondary antibody and reduce the signal.*

How to Prepare Serum Blocking Buffer:

A proven formula used in many immunoassays is a 5% (v/v) normal serum solution in Phosphate Buffered Saline (PBS) with Tween® 20 detergent.

- 1) Add serum and mix with diluent buffer (PBS w/ 0.05% Tween-20). IR Cat# BU-117
- 2) Use immediately or store at 2-8 °C or colder.

Serum Volumes Guide:

Serum Volume	2 ml	5 ml	10 ml
Diluent Buffer Volume (IR Cat # BU-117)	40 ml	100 ml	200 ml
Total Volume	42 ml	105 ml	210 ml

**ImmunoReagents supplies serum in 2, 5, and 10 ml units. Serum Diluent Buffer is supplied in 500ml units.*