Improved Multiplexing with Simple Western using a 20X Rabbit HRP Conjugate

Multiplexing doubles your data count

Multiplexing two targets in one capillary with Simple Western® doubles the number of data points per sample and increases the data quantitation accuracy when you normalize to biological loading control or system control.

When you're ready to start multiplexing, first optimize the primary antibodies separately to find the saturating dilution for each. Then combine them in Antibody Diluent 2 to detect the two targets in the same capillary. If the primary antibodies are raised in two different host species, you'll also need to combine two different anti-species secondary antibodies. But mixing Ready-To-Use (RTU) secondary antibodies will dilute them and cause the signal to decrease or become more variable because they're no longer saturating. We've got the situation under control with the 20X Anti-Rabbit HRP Conjugate.

Recover signal loss

The 20X Anti-Rabbit HRP Conjugate (PN 043-426) is a concentrated version of our RTU Anti-Rabbit Secondary Antibody (PN 042-206) for equivalent performance. It's developed to help multiplex a rabbit primary antibody and should only be diluted to 1X with a ProteinSimple RTU secondary antibody. Simply mix the 20X Anti-Rabbit HRP Conjugate with the RTU secondary at a 1:20 ratio, then transfer the mixture to a Wes®, Sally Sue®, or Peggy Sue® plate.

When we multiplexed ERK1/2 and STAT3 in 0.1 mg/mL HeLa lysate and combined ProteinSimple RTU rabbit and RTU mouse secondary antibodies directly, all peak areas decreased approximately 38% compared to the individually run samples (**Figure 1, left**). Replacing the RTU Anti-Rabbit Secondary with the 20X Anti-Rabbit HRP Conjugate recovered this peak area loss. (**Figure 1, right**).

Next time you multiplex a rabbit primary with an antibody raised in different species, use the new 20X Anti-Rabbit HRP Conjugate for the best results.

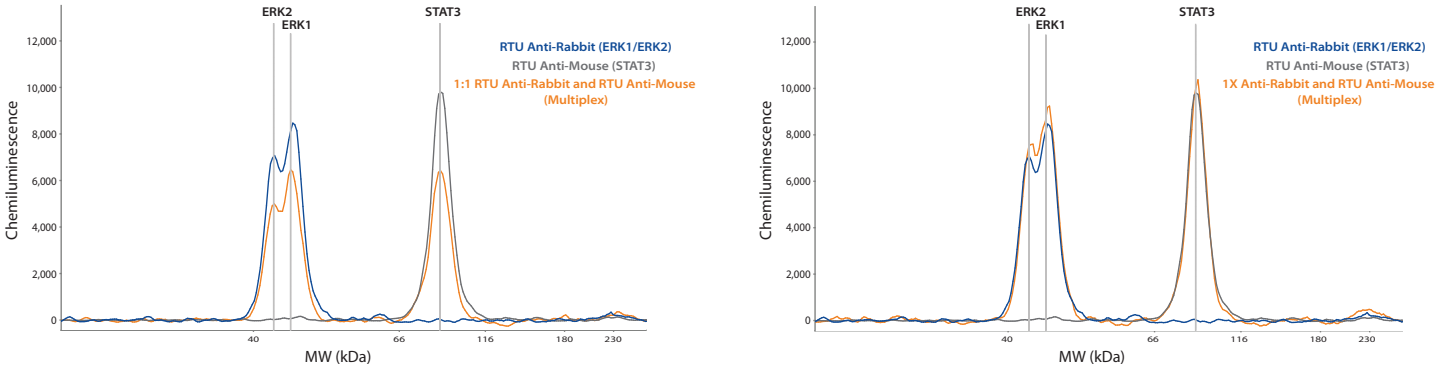

FIGURE 1. Multiplexing proteins using primary antibodies from different host species resulted in a significant decrease when mixing RTU secondary antibodies 1:1 (left). Mixing a 20X Anti-Rabbit HRP Conjugate into the RTU Anti-Mouse Secondary at a 1:20 ratio gave equivalent results to individually run samples (right).

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Toll-free: (888) 607-9692
Tel: (408) 510-5500
info@proteinsimple.com
proteinsimple.com

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