ROCKLAND™
antibodies & assays

ADVANCING LIFE SCIENCE TO FOSTER A BETTER WORLD

Revitablot™ Stripping Buffer

Test with Secondary HRP-conjugated, Dylight®-conjugated, and IRDye®-conjugated Antibodies

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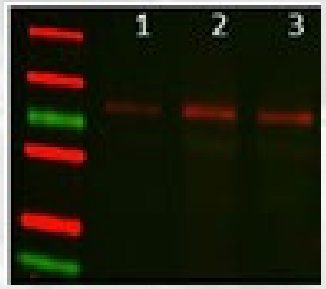
Revitablot™ Product Specifics

- **Fast |** Strips most proteins in as little as 10 minutes
- **Efficient |** Minimal optimization required
- **Easy |** Ambient incubation requires little intervention
- **Safe |** Non-toxic materials
- **Multiple Applications |** Effective for both Nitrocellulose and PVDF membranes
- **Multi-Use |** Can be used to strip at least four times

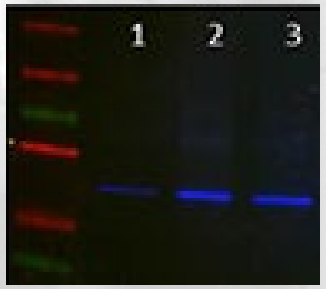
Efficiency in WB



(650 nm Red, 549 nm Green)

60 kDa

Anti-AKT pS473 (MAb)
DyLight™ 649 Conjugated



(650 nm Red, 488 nm Blue)

42 kDa

Anti-Beta-Actin pS473 (PAb)
DyLight™ 488 Conjugated

LANE 1:	7.5 µg HCT – 116 cell lysates
LANE 2:	22.5 µg HCT – 116 cell lysates
LANE 3:	15 µg HCT – 116 cell lysates

Colorectal carcinoma HCT-116 whole cell lysates were analyzed on a SDS-PAGE 4–20% gradient gel (Bio-Rad) and transferred to nitrocellulose membranes.

The lysates were followed by blocking with 5% BLOTTO (#B501-0500) for 1 hour at room temperature.

The membranes were first probed with Anti-AKT pS473 (MOUSE)(#200-343-268) at a dilution of 1:1,000 overnight at 4°C. Imaging was performed after drying membranes at 650 nm.

Membranes were then incubated with Revitablot™ for 5 minutes at room temperature, followed by re-blocking with 5% BLOTTO for 2 hours at room temperature.

Secondary probing was performed with Anti-Beta-Actin (RABBIT) (#CUST17) at a dilution of 1:1,000 for 4 hours at room temperature. Membranes were then dried, imaged at 488 nm and 650 nm.

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HRP-Conjugated & Fluorescent-Conjugated Antibodies Protocol

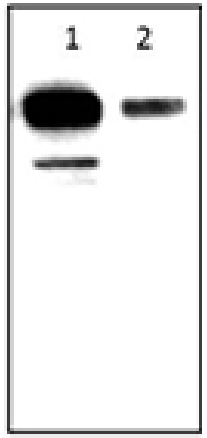
1. Image for the primary target and then wash blot for at least 10 minutes in 1x TBS-T on an orbital shaker at room temperature. This process removes any remaining HRP substrate.
2. Decant TBS-T and apply 10 mL of Revitablot ™
   Note: Volume depends on size of blot; apply enough buffer to sufficiently cover the membrane. Incubate from 5–10 minutes (user determined) on an orbital shaker at room temperature.
3. Decant Revitablot™ buffer and wash blot for at least 10 minutes in 1X TBS-T as in Step 1.
4. Decant TBS-T and block blot in 5–10 mL of 5% BLOTTO (#B501-0500) for 2 hours on an orbital shaker at room temperature.

HRP-Conjugated & Fluorescent-Conjugated Antibodies Protocol (Cont.)

5. Decant the BLOTTO and apply the next primary antibody target. Incubate for the optimum time and conditions per provided instructions.
6. Complete the Western blot procedure (washing, application of secondary antibody, substrate addition, and imaging).
   Note: Blots may be stripped up to four times for HRP-Conjugated antibodies and up to two times for fluorescent-conjugated antibodies.

Revitablot vs. Competitor

Revitablot™



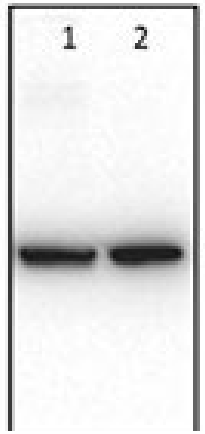
95 kDa

Competitor

Incubation Blots with Stripping Buffers

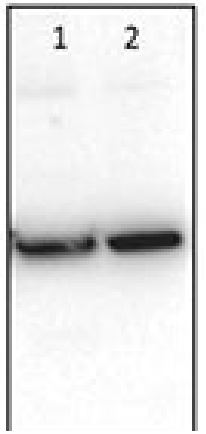
(20 minutes)

Revitablot™



95 kDa

Competitor



Initial Target
STAT5
(#600-401-A44)

LANE 1:	70 µg
LANE 2:	10 µg

Re-Probe for
α-Tubulin
(#200-301-880)

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THANK YOU

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