

Rabbit IgG SmartBlot IP/Western Blot Reagents

Rabbit IgG SmartBlot is immunoblotting detection reagents, which react strongly with native rabbit IgG primary antibodies used for detecting specific antigens on Western blots without binding to the reduced and denatured rabbit IgG heavy chain band (50 kD) and light chain band (25 kD), which come from the rabbit primary antibody used for immunoprecipitating the antigens of interest from cell lysates or protein mixtures. Therefore, by using our Rabbit IgG SmartBlot, detection of antigens with molecular weights near 50 kD or 25 kD is not obscured by large amounts of reduced and denatured rabbit IgG heavy chains and light chain on the blots.

Highlights:

EFFECTIVE — Dramatically reduces the noise of heavy chain and light chain observed when Western Blotting immunoprecipitated proteins.

SIMPLE — No changes to your existing immunoprecipitation and western blot protocols.

COMPATIBLE — Use of anti-rabbit IgG beads for immunoprecipitation.

Products:

Rabbit IgG SmartBlot-HRP: va-4000-001, 50ul
Rabbit IgG SmartBlot-AP: va-4000-002, 50ul
Rabbit IgG SmartBlot-Biotin: va-4000-003, 50ul

Store at 4° C and stable for six months from the date of shipment.

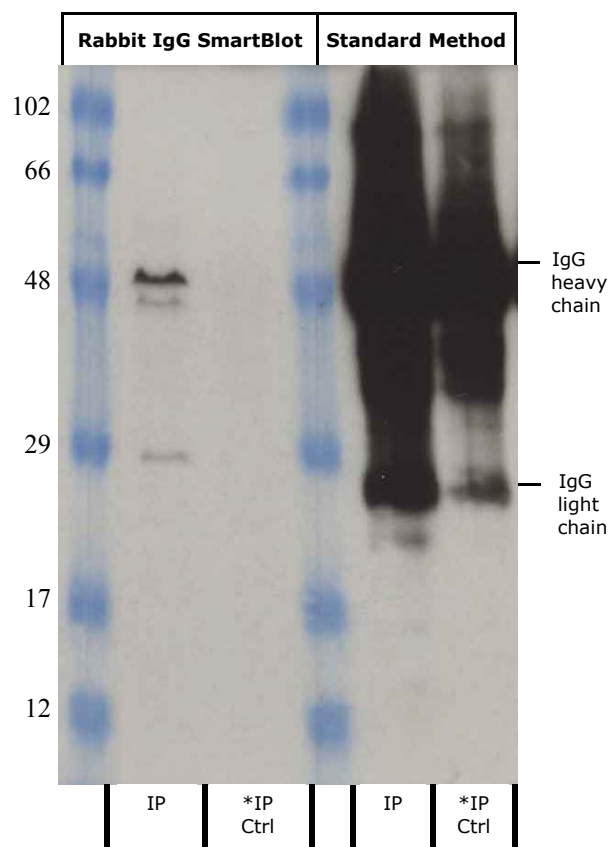
Related Products:

Goat IgG SmartBlot-HRP: va-6000-001
Goat IgG SmartBlot-AP: va-6000-002
Goat IgG SmartBlot-Biotin: va-6000-003
Western Blot Signal Enhancer: va-100
Blocking Reagent: va-99
Luminol Reagent: va-98

Research Use:

For research use only, not for use in diagnostic procedures.

Data:



A431 cell lysates are immunoprecipitated and western blotted with rabbit anti-p53 polyclonal antibodies, then detected with either Rabbit IgG SmartBot-HRP or conventional HRP conjugated anti-goat secondary antibody (Standard Method).

* Note: IP Ctrl lanes have no cell lysates.

Procedure (Use Rabbit IgG SmartBlot-HRP as an example):

Preparation of Immunoprecipitated Sample for SDS-PAGE

Follow standard procedure to run immunoprecipitation with Anti-rabbit IgG Beads and resuspend the immunoprecipitated Beads in fresh Laemmli Buffer with reducing reagent

Western Blot

Prepare sample as indicated above, run on SDS-PAGE and transfer the separated proteins to membrane.

Block the membrane with 5% milk in TBST

Incubate the membrane with rabbit IgG primary antibody diluted in 5% milk in Western Blot Enhancer at room temperature for 1 hour or overnight at 4°C with gentle shaking

Wash the membrane with TBST 3x 5min

Incubate the membrane with Rabbit IgG SmartBlot at a recommended 1:1,000 dilution in 5% milk in Western Blot Enhancer for 1 hour at room temperature with gentle shaking.

Wash the membrane with TBST 3x 5min and TBS 1x 5min

Develop the blot using a chemiluminescent-HRP substrate.
(*AP substrate if Rabbit IgG SmartBlot-AP is used or avidin detection reagent if Rabbit IgG SmartBlot-Biotin is used*)

Expose the membrane to X-ray film for the desired time. A 1 minute exposure is a suggested starting time and the exposure time can be shortened or lengthened as desired.