

# Produktinformation



Forschungsprodukte & Biochemikalien



Zellkultur & Verbrauchsmaterial



Diagnostik & molekulare Diagnostik



Laborgeräte & Service

Weitere Information auf den folgenden Seiten! See the following pages for more information!



## Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

## Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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#### **Datasheet for 18-8817-33**

## Mouse TrueBlot® ULTRA: Anti-Mouse Ig HRP

### **Overview**

Description:	Mouse TrueBlot® ULTRA: Anti-Mouse Ig HRP - 18-8817-33
Item No.:	18-8817-33
Size:	200 μL
Applications:	ELISA, IP, WB, Biochemical Assay, ChIP, IF
Reactivity:	Mouse
Host Species:	Rat

### **Product Details**

Background:	Mouse IgG TrueBlot® ULTRA is a unique Anti-Mouse IgG monoclonal secondary antibody.
Ducing Curra.	Widase is a macbiol of this is a anique with induse is a monocional secondary antibody

Mouse IgG TrueBlot® ULTRA enables detection of immunoblotted target protein bands, without hindrance by interfering immunoprecipitating immunoglobulin heavy and light chains. It is easy to generate publication-quality IP/Western blot data with Mouse IgG TrueBlot® ULTRA, simply substitute the conventional Anti-Mouse IgG blotting reagent with Mouse IgG TrueBlot® ULTRA and follow the prescribed protocol for sample preparation and immunoblotting. Mouse IgG TrueBlot® ULTRA is ideal for use in protocols involving immunoblotting of immunoprecipitated proteins. Mouse IgG TrueBlot® ULTRA preferentially detects the non-reduced form of mouse IgG (IgG1, IgG2a, IgG2b, IgG3) over the reduced, SDS-denatured form of IgG. When the immunoprecipitate is fully reduced immediately prior to SDS-gel electrophoresis, reactivity of Mouse IgG TrueBlot® ULTRA with the 55 kDa heavy chains and the 23 kDa light chains of the immunoprecipitating antibody is minimized thereby eliminating interference by the heavy and light chains of the immunoprecipitating antibody in IP/immunoblot applications. Applications include studies examining post-translational modification (e.g., phosphorylation or acetylation) or protein-protein interactions.

	or protein protein meradions.
Synonyms:	Anti-Mouse IgG HRP, TrueBlot, HRP TrueBlot ULTRA, Peroxidase TrueBlot, TrueBlot for IP/WB, TrueBlot for immunoprecipitation, TrueBlot for western blotting
<b>Host Species:</b>	Rat
Conjugate:	Peroxidase (HRP) ULTRA
Clonality:	Monoclonal
Clone ID:	eB144
Format:	IgG

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## **Target Details**

Reactivity:	Mouse
Purity/Specificity:	Mouse TrueBlot® ULTRA Antibody Peroxidase Conjugate was prepared from tissue culture supernatant by Protein G affinity chromatography. Assay by immunoelectrophoresis resulted in a single precipitin arc against Anti-Mouse Serum. Reactivity is observed against native Mouse IgG by both Western blot and ELISA.
Relevant Links:	TrueBlot HRP Product Protocols
	TrueBlot IP Set Protocol

# **Application Details**

• •	
<b>Tested Applications:</b>	ELISA, IP, WB
Suggested Applications:	Biochemical Assay, ChIP, IF (Based on references)
Application Note:	Mouse TrueBlot® ULTRA has been tested in ELISA, IP, and Western blot and may also be used for detection in immunoblotting assays that do not employ immunoprecipitation. Mouse IgG TrueBlot® ULTRA is provided as 1000X solution. To achieve best results when detecting mouse IgG1 subtypes, we recommend performing a dot blot or titration to determine the optimal dilution factor for your desired application. All recommended dilutions for listed applications are intended as an initial recommendation, specific conditions for each protein and antibody combination should be specifically optimized by the end user. In order to conserve reagent, we recommend incubating the blots from minigels in sealed bags (removing as much air as possible) with minimal volume (2-3 mLs). If used conservatively at 2.5mls/blot will yield enough reagent for 8 blots. Note that there are three key procedural considerations: 1. Protein A or G beads may be used with the mouse, goat and sheep TrueBlot secondaries, but not with the rabbit TrueBlot secondary. Use of protein A or G beads with the rabbit TrueBlot will result in contaminating bands. 2. Immunoprecipitate should be completely reduced. 3. BLOTTO/Milk should be used as the blocking protein for the immunoblot.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
IF:	User Optimized
IP:	User Optimized
WB:	1:1000

### **Formulation**

Physical State: Liquid (sterile filtered)

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Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Gentamicin Sulfate
Stabilizer:	0.1 mg/ml Bovine Serum Albumin (BSA) - IgG and Protease free, 50% (v/v) Glycerol

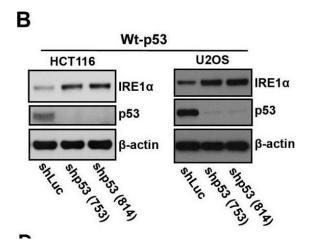
# **Shipping & Handling**

Shipping Condition:	Wet Ice
Storage Condition:	Store at -20°C. This product is guaranteed for 6 months upon receipt, when handled and stored as instructed.
Expiration:	Expiration date is six (6) months from date of receipt.

## **Images**

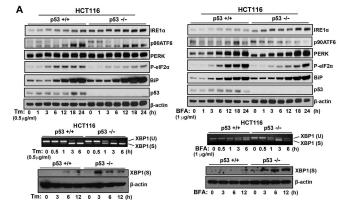
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#### **Western Incubation Box**

IRE1α expression is regulated by p53. A.) Western blot analysis of the expression of endogenous IRE1α in 23 human cancer cell lines. Cell lines were grouped according to expression of wild-type or mutant p53 as indicated. (A well between wt-p53 and mutant-p53 cell lines was cut, from the gel as indicated by a black line, due to the controversial p53 status of the cell line). Right panel: The intensities of the IRE1 $\alpha$  bands (left panel) are expressed relative to those of β-actin. Values shown are the mean ± standard deviation (s.d.). The P value was calculated using two-way ANOVA. B.) Downregulation of p53 expression induces increased expression of IRE1α. HCT116 p53+/+ and U2OS cells were transfected with shLuc, shp53 (753), or shp53 (814), and selected using puromycin. Whole cell lysates of a pool of transfectants were analyzed using western blotting with the indicated antibodies. C.) Overexpression of wild-type p53 inhibits IRE1α expression in mutant-p53 cell lines. Cell lysates, prepared 48 h after transfection with wild-type p53, were analyzed for the expression of indicated proteins. D.) Mutant p53 proteins do not inhibit IRE1α expression. Cell lysates were prepared from cells transfected with p53-G245S, p53-R248W, p53-249S, and p53-R273H expression vectors or from cells that constitutively expressed wild-type p53 and were analyzed for the expression of the indicated proteins. Figure provided by CiteAb. Source: Oncotarget, PMID: 26254280.

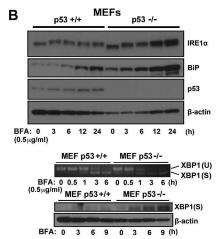


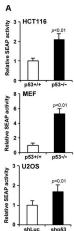
### **Western Blot**

ER stress response in p53-deficient or knockdown cells. A.) HCT116 p53+/+ or HCT116 p53-/– cells, B. MEF p53+/+ or MEF p53-/– cells, and C. U2OS shLuc or U2OS shp53 cells were incubated with Tm (0.5  $\mu$ g/mL) or BFA (1  $\mu$ g/mL) for the times indicated. Cell lysates were analyzed using western blotting with the indicated antibodies. The blot was cut based on the size of proteins or stripped. Total RNAs were extracted and subjected to RT-PCR analysis using specific primer sets for XBP1(U) and XBP1(S). Cell lysates were analyzed using western blotting with indicated antibodies. Figure provided by CiteAb. Source: Oncotarget, PMID: 26254280.

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#### **Western Blot**

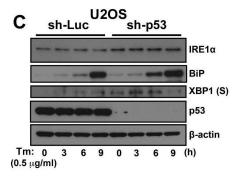
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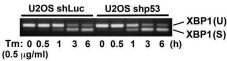
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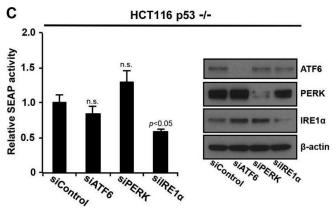
p53 deficiency increases secretory the function of the ER through the IRE1 $\alpha$ /XBP1 pathway. A.) HCT116 p53+/+ or HCT116 p53-/- cells, MEF p53+/+ or MEF p53-/- cells, and U2OS shLuc or U2OS shp53 cells expressing secreted embryonic alkaline phosphatase (SEAP) were transduced with a pSEAP2 control vector and washed 24 h after transduction. The medium was then changed, and the cells were cultured for another 6 h. Culture media were analyzed for SEAP activity, and luminescence was normalized to cell number. The transfection efficiencies of HCT116 p53+/+ and HCT116 p53-/- cells were approximately 80% each (data not shown). B.) Overexpression of wild-type p53 inhibited SEAP activity. SEAP activities of cells that constitutively expressed the indicated p53 molecules were analyzed using the same procedure described in (A). C.) HCT116 p53-/- cells that expressed SEAP were transfected with siControl, siATF6, siPERK, or siIRE1 $\alpha$ , cultured for 24 h, and following a change of medium, the cells were cultured for another 6 h. Whole cell lysates were analyzed using western blotting with the indicated antibodies, and culture supernatants were analyzed for SEAP activity. Values shown are the mean  $\pm$  s.d. of three different experiments simultaneously measured. The P value was calculated using two-way ANOVA. Figure provided by CiteAb. Source: Oncotarget, PMID: 26254280.

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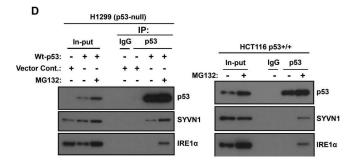
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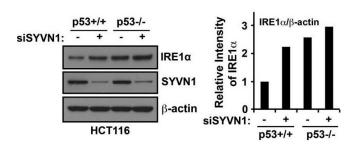
#### **Western Blot**

Synoviolin promotes IRE1α degradation in a wild-type p53dependent manner. A.) SYVN1 suppresses IRE1α protein expression in wild-type p53 cells. HCT116 p53+/+ or HCT116 p53-/- cells were transfected with siControl (-) or siSYVN1 (+) and cultured for 24 h. Cell lysates were analyzed using western blotting with indicated the antibodies (left panel). The intensities of the SYVN1 bands were quantified. The levels of SYVN1 are reported relative to those of β-actin (right panel). The blot was cut based on the size of proteins or stripped and reprobed. B.) IRE1 $\alpha$  and SYVN1 interaction is suppressed in p53-deficient cells. Proteins were cross-linked with DSP before protein extraction. Coimmunoprecipitation was performed with cell lysate using an IRE1α or an SYVN1 antibody. C.) SYVN1 interacts with wild-type p53. H1299 cells transiently expressed wild-type p53, p53-R248S, or p53-R273H. Coimmunoprecipitation experiments were performed using the anti-p53 antibody. D.) p53-SYVN1-IRE1 $\alpha$  complex is observed by treatment with proteasome inhibitor. H1299 cells transiently expressing wild-type p53 (left panel) or HCT116 p53+/+ (right panel) cells were treated with 50 µM MG132 for 3 h. Coimmunoprecipitation experiments were performed using the anti-p53 antibody. Figure provided by CiteAb. Source: Oncotarget, PMID: 26254280.

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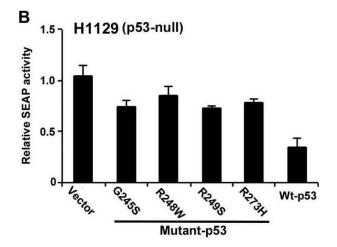


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Synoviolin promotes IRE1α degradation in a wild-type p53dependent manner. A.) SYVN1 suppresses IRE1α protein expression in wild-type p53 cells. HCT116 p53+/+ or HCT116 p53-/- cells were transfected with siControl (-) or siSYVN1 (+) and cultured for 24 h. Cell lysates were analyzed using western blotting with indicated the antibodies (left panel). The intensities of the SYVN1 bands were quantified. The levels of SYVN1 are reported relative to those of β-actin (right panel). The blot was cut based on the size of proteins or stripped and reprobed. B.) IRE1 $\alpha$  and SYVN1 interaction is suppressed in p53-deficient cells. Proteins were cross-linked with DSP before protein extraction. Coimmunoprecipitation was performed with cell lysate using an IRE1 $\alpha$  or an SYVN1 antibody. C.) SYVN1 interacts with wild-type p53. H1299 cells transiently expressed wild-type p53, p53-R248S, or p53-R273H. Coimmunoprecipitation experiments were performed using the anti-p53 antibody. D.) p53-SYVN1-IRE1 $\alpha$  complex is observed by treatment with proteasome inhibitor. H1299 cells transiently expressing wild-type p53 (left panel) or HCT116 p53+/+ (right panel) cells were treated with 50 µM MG132 for 3 h. Coimmunoprecipitation experiments were performed using the anti-p53 antibody. Figure provided by CiteAb. Source: Oncotarget, PMID: 26254280.

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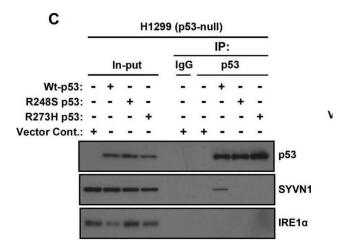
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#### Mutant-p53 **AU565** SNU1040 HCT116 p53 -/-IRE1α IRE1α IRE1α p53 p53 p53 MDM2 MDM<sub>2</sub> MDM2 β-actin β-actin β-actin

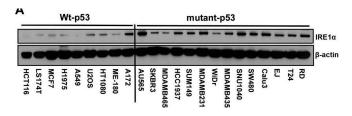
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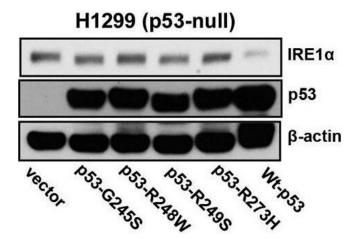


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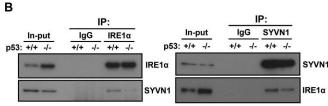


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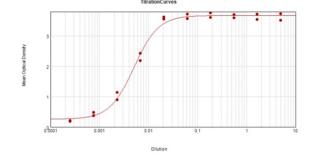


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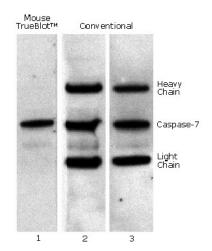
#### **ELISA**

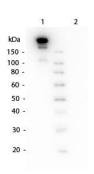
ELISA Results of Mouse TrueBlot® ULTRA: Anti-Mouse Ig HRP. Each well was coated in duplicate with 1.0 µg of Mouse TrueBlot® ULTRA: Anti-Mouse Ig HRP (red line). The starting dilution of antibody was 5µg/ml and the X-axis represents the Log10 of a 3-fold dilution. The titer is 199,000. This titration is a 4-parameter curve fit where the IC50 is defined as the titer of the antibody. Assay performed using TMB-1000 substrate.



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#### **Western Blot**

Mouse TrueBlot® IP / Western Blot: Caspase 7 was immunoprecipitated from 0.5 ml of 1x10e7 Jurkat cells/ml with 5 ug mouse anti-human Caspase 7. Precipitate from 1x10e6 cells was subjected to electrophoresis, transferred to an PVDF membrane, and Western blotted with anti-Caspase 7 using Mouse TrueBlot® ULTRA: Anti-Mouse Ig HRP (Lane 1) or conventional HRP-conjugated anti-mouse antibody (Lane 2) - note the detection of the heavy and light chains of the immunoprecipitating antibody in Lane 2 but not in Lane 1. When Lane 1 is re-immunoblotted using conventional HRPconjugated anti-mouse polyclonal antibody (Lane 3), the heavy and light chains are now detected, confirming that although the immunoprecipitating heavy and light chains are present, Mouse TrueBlot® ULTRA: Anti-Mouse Ig HRP detects only native antibody and not denatured heavy and light chains.

#### **Western Blot**

Western Blot of Mouse TrueBlot® ULTRA: Anti-Mouse Ig HRP. Lane 1: Mouse IgG, non-denatured. Center Lane:  $5~\mu L$  Opal Pre-stained Ladder (p/n MB210-0500). Lane 2: Mouse IgG, denatured. Load: 50~ng. Primary antibody: none. Secondary antibody: Mouse TrueBlot® ULTRA: Anti-Mouse Ig HRP at 1:1,000 for 60~min at RT. Block: MB-070 for 30~min at RT. Predicted/Observed size: >160 kDa non-denatured for Mouse IgG.

### References

- Lin LL et al. SEL1L-HRD1 interaction is required to form a functional HRD1 ERAD complex. Nat Commun. (2024)
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