abcam

ab57653

Anti-RRM2 antibody [1E1]

Key facts

Isotype	IgG1
Host species	Mouse
Storage buffer	pH: 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	Recombinant Fragment Protein within Human RRM2 aa 1-150. The exact immunogen used to generate this antibody is proprietary information. Database link P31350
Clone number	1E1
Purification technique	Affinity purification Protein A
Light chain type	kappa

Reactivity data

Flow Cyt

Tested

Species	Human
Dilution info	-
Notes	ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

WB

Tested

Species	Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Target data

See full target information RRM2

Function	Provides the precursors necessary for DNA synthesis. Catalyzes the biosynthesis of deoxyribonucleotides from the corresponding ribonucleotides. Inhibits Wnt signaling.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C

Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

This product was changed from ascites to tissue culture supernatant on 12/3/19. Please note that the dilutions may need to be adjusted accordingly. If you have any questions, please do not hesitate to contact our scientific support team.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	RRM2 also known as Ribonucleotide Reductase Regulatory Subunit M2 is an important component of the ribonucleotide reductase enzyme complex which plays a significant part in DNA synthesis. This protein helps convert ribonucleotides into deoxyribonucleotides. RRM2 is approximately 45 kDa in size. Its expression occurs mostly in proliferating cells with high levels detected in the cytoplasm and nucleus. The expression level of RRM2 can vary significantly across different tissues correlating closely with cell division rates.
Biological function summary	The ribonucleotide reductase enzyme facilitates the critical function of DNA replication and repair. RRM2 forms a complex with RRM1 the large subunit necessary for enzyme activity. This complex regulates the balance of deoxyribonucleotide pools necessary for DNA synthesis. Variations or dysregulations in the activity of RRM2 can influence genetic stability and cell proliferation emphasizing its importance in cellular function.
Pathways	Ribonucleotide reductase and RRM2 participate significantly in the DNA synthesis and repair pathway. It is pivotal for maintaining DNA fidelity during replication. RRM2 actions relate to pathways involving other DNA replication proteins such as RRM1 and the replication factor C family. The synthesis pathway necessitates close cooperation between these proteins to ensure proper DNA replication and cell cycle progression.
Associated diseases and disorders	Research has linked RRM2 to cancer and viral infections. Overexpression or mutations in the RRM2 gene may contribute to cancer development as seen in various tumor types indicating it as a potential target for cancer therapeutics. Moreover some viruses may hijack the host's RRM2 to facilitate their own replication. Targeting the RRM2 protein could potentially lead to novel treatment approaches in these diseases connecting it with other proteins like RRM1 which also share roles in tumor progression and viral replication.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

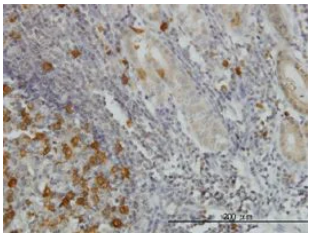
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

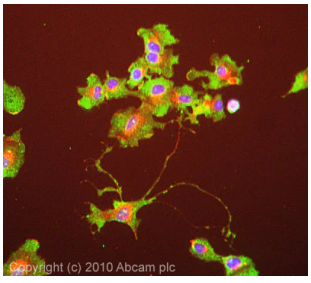
7 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RRM2 antibody [1E1] (ab57653)

RRM2 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-RRM2 antibody

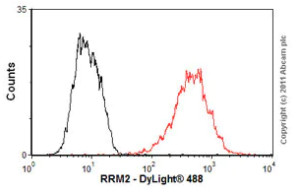
RRM2 antibody (ab57653) used in immunohistochemistry at 5ug/ml on formalin fixed and paraffin embedded human stomach.
This image was generated using the ascites version of the product.



Immunocytochemistry/ Immunofluorescence - Anti-RRM2 antibody [1E1] (ab57653)

RRM2 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-RRM2 antibody

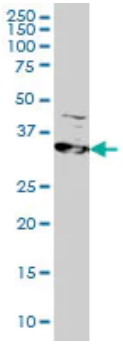
ICC/IF image of ab57653 stained HepG2 cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab57653, 10µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.
This image was generated using the ascites version of the product.



Flow Cytometry - Anti-RRM2 antibody [1E1] (ab57653)

RRM2 Flow Cytometry staining using mouse Anti-RRM2 antibody

Overlay histogram showing HepG2 cells stained with ab57653 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab57653, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.
This image was generated using the ascites version of the product.



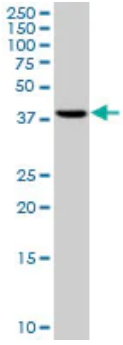
Western blot - Anti-RRM2 antibody [1E1] (ab57653)

RRM2 Western blot staining using mouse Anti-RRM2 antibody

RRM2 antibody (ab57653) at 1ug/lane + HeLa cell lysate at 25ug/lane.
This image was generated using the ascites version of the product.

All lanes:
Western blot - Anti-RRM2 antibody [1E1] (ab57653)

Predicted band size: 45 kDa



Western blot - Anti-RRM2 antibody [1E1] (ab57653)

RRM2 Western blot staining of HepG2 (human liver hepatocellular carcinoma cell line) cell lysate using mouse Anti-RRM2 antibody

This image was generated using the ascites version of the product.
All lanes:
Western blot - Anti-RRM2 antibody [1E1] (ab57653) at 1 µg/mL

All lanes:
HepG2 (human liver hepatocellular carcinoma cell line) cell lysate

Predicted band size: 45 kDa

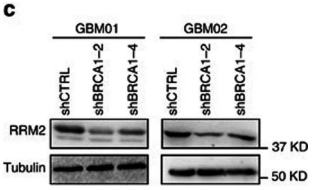


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Western blot - Anti-RRM2 antibody [1E1] (ab57653)

RRM2 Western blot staining using mouse Anti-RRM2 antibody

Western Blotting using Anti-RRM2 antibody [1E1], ab57653. Publication image from Bartek, J. et al., 2016, Nat Commun, 27845331. Legend direct from paper.

BRCA1 loss impedes replication fork progression by down-regulating RRM2 in GBM cells.(a) DNA fibre assay measuring the replication fork progression speed in GBM cells (GBM01 and GBM02) transduced with shCTRL or two independent BRCA1-targeting shRNAs (shBRCA1-2 and shBRCA1-4). See also Table 1. (b) Replication fork recovery assay showing the quantification of CldU tract length in GBM01 cells transduced with shCTRL or 2 non-overlapping BRCA1-targeting shRNAs (shBRCA1-2 and shBRCA1-4) and treated or not with 2 mM HU (4 h) prior CldU labelling. See also Table 2; Supplementary Table 1. (c) Immunoblot analysis of RRM2 protein levels in GBM cells transduced with shCTRL or two independent BRCA1-targeting shRNAs (shBRCA1-2 and shBRCA1-

4). (d,e) RT-qPCR analysis of BRCA1 and RRM2 mRNA levels in GBM cells transduced with shCTRL or two independent BRCA1-targeting shRNAs (shBRCA1-2 and shBRCA1-4). (f) FACS analysis of RRM2 protein level changes throughout cell cycle in GBM cells (GBM01) transduced with shCTRL or two independent BRCA1-targeting shRNAs (shBRCA1-2 and shBRCA1-4). (g) Partial sequence of the human RRM2 promoter (GenBank accession number AY032750)33, which was used to design Chip primers: P1 primer forward (F)/reverse (R) and P2 primer forward (F)/reverse (R). Positions are numbered from the downstream transcription initiation site (+1). Putative binding sites for transcription factors are color-coded and identified above the sequence. (h) Chip immunoprecipitation of BRCA1 binding RRM2 promoter in GBM01-03 cells using primer set P1 and P2. (i) Chip immunoprecipitation of BRCA1 binding RRM2 promoter in NHA-DRB and BJ cells using primer set P1 and P2. (j) Chip immunoprecipitation of BRCA1 binding RRM2 promoter in PC3, HELA, OVCAR and Cal51 cells using primer set P1 and P2. Statistical significance was calculated by one-way ANOVA and Tukey's multiple comparisons test in d–f,h–j or Student's t test (a,b) and all data are shown as means±s.d. and performed as technical triplicates. (*P<0.05, **P<0.005, ***P<0.005, ****P<0.0001; NS represents non-significance).

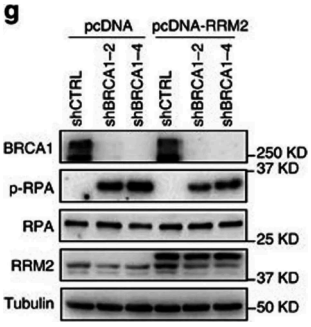


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Western blot - Anti-RRM2 antibody [1E1] (ab57653)

RRM2 Western blot staining using mouse Anti-RRM2 antibody

Western Blotting using Anti-RRM2 antibody [1E1], ab57653. Publication image from Bartek, J. et al., 2016, Nat Commun, 27845331. Legend direct from paper.

BRCA1 regulates RRM2 expression via E2F1 and RRM2 overexpression rescues BRCA1-loss phenotype in GBM cells.(a) Luciferase assay of transcriptional activation of the RRM2 promoter in GBM01 transduced with shCTRL or shBRCA1-2/shBRCA1-4. (b) Luciferase assay of transcriptional activation of the RRM2 promoter in GBM01 after siRNA-mediated knockdown of Sp1; AP-1 and E2F1 in comparison to BRCA1. (c) Luciferase assay of transcriptional activation of the RRM2 promoter in GBM01 after siRNA-mediated knockdown of BRCA1 and E2F1 alone or on combination. (d) Chip immunoprecipitation of E2F1 binding RRM2 promoter in GBM01 cells using primer set P1 and P2. (e) Chip immunoprecipitation of BRCA1 binding RRM2 promoter in GBM01 cells transfected with siCTRL and siE2F1 using primer set P1 and P2. (f) DNA fibre assay measuring the replication fork progression speed in GBM cells lacking BRCA1 (shBRCA1-2 and shBRCA1-4) or not (shCTRL) transfected with either control vector (pcDNA) or vector expressing RRM2 (pcDNA-RRM2). See also Table 3. (g) Cell viability of GBM cells lacking BRCA1 (shBRCA1-2 and shBRCA1-4) or not (shCTRL) transfected with either control vector (pcDNA) or vector expressing RRM2 (pcDNA-RRM2). (h) Immunoblot analysis of BRCA1, p-RPA, RPA and RRM2 in GBM cells lacking BRCA1 (shBRCA1-2 and shBRCA1-4) or not (shCTRL) transfected with either control vector (pcDNA) or vector expressing RRM2 (pcDNA-RRM2). (i) Viability-based assessment of EC50 triapine concentrations in GBM01, GBM02 and GBM03 cells. (j) DNA fibre assay measuring the replication fork progression speed in GBM cells treated with DMSO or EC50 triapine. See also Table 4. Statistical significance was calculated by one-way ANOVA and Tukey's multiple comparisons test in a–d,f,h or Student's t test (e,i) and all data are shown as means ±s.d. and performed as technical triplicates. (*P<0.05, **P<0.005, ***P<0.005, ****P<0.0001; NS represents non-significance).

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