Human RBM3 ELISA Assay Kit

REF BTRBM-001

For quantitative determination of human RNA-binding protein 3

96 Determinations

2°C – 8 °C

For research use only
Not for diagnostic use

Contents

Short Review	3
Intended Use	4
Test principle	5
Safety warning and precautions	5
Storage	6
Components of the assay system	6
Sample preparation and storage	7
Critical parameters	7
Preparation of reagents	8
Assay protocol	8
Protocol summary	9
Scheme of the plate	10
Data processing	10
Additional information	11
Specificity	11
Sensitivity	11
Linearity	11
Recovery	11
Reproducibility	12
Troubleshooting	13
Related Products	14

BioTeZ Berlin-Buch GmbH
Robert-Rössle-Str. 10, Haus 72
13125 Berlin
Tel.: ++49 (0)30-9489 3322
Fax: ++49 (0)30-949 2008
e-mail: pro@biotez.de
http://www.biotez.de

This manual is valid from May, 2016

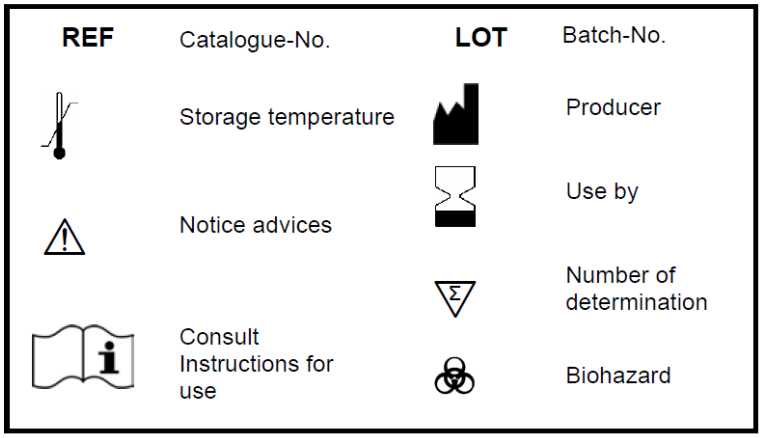
Short Review

The RNA-binding protein 3 (RBM3) is a member of the glycine-rich RNA-binding protein family. The exact role of the cold shock protein RBM3 is not yet clear [2, 6]. The role of mRNA-binding proteins can be attributed to a regulation of mRNA splicing, stability, transport and ultimately the translation of mRNA into proteins. RBM3 consists of 157 amino acids with a predicted mass of 17 kD (1, 2) and is one of the few proteins that are upregulated at low temperature [3-5]. It is known that RBM3 acts especially in cellular stress (eg. hypothermia) as mRNA chaperones and protein translation stabilizer [1, 4, 5]. Chip et al. were able to show that RBM3 has anti-apoptotic properties in neurons [7] and recent studies show that RBM3 mediates structural plasticity and protective effects of neurodegeneration (8). For these reasons, RBM3 may play an important role as biomarker for many neurodegenerative diseases and could be used to monitor the effectiveness of therapeutic hypothermia. Several studies report RBM3 as prognostic marker in cancer or in linking stress-regulated RNA splicing to tumorgenesis. Low nuclear expression of RBM3 has been found to be associated with poor prognosis in several cancer forms e.g. breast, ovarian, colorectal, prostate cancer and malignant melanoma (9-14).

References

1. Danno, S., et al., Increased transcript level of RBM3, a member of the glycine-rich RNA-binding protein family, in human cells in response to cold stress. Biochemical and Biophysical Research Communications, 1997. 236(3): p. 804-807
2. Derry, J.M., J.A. Kerns, and U. Francke, RBM3, a novel human gene in Xp11. 23 with a putative RNA-binding domain. Human molecular genetics, 1995. 4(12): p. 2307-2311.
3. Dresios, J., et al., Cold stress-induced protein Rbm3 binds 60S ribosomal subunits, alters microRNA levels, and enhances global protein synthesis. Proceedings of the National Academy of Sciences of the United States of America, 2005. 102(6): p. 1865-1870.
4. Nishiyama, H., et al., Cloning and characterization of human CIRP (cold-inducible RNA-binding protein) cDNA and chromosomal assignment of the gene. Gene, 1997. 204(1-2): p. 115-120
5. Smart, F., et al., Two isoforms of the cold-inducible mRNA-binding protein RBM3 localize to dendrites and promote translation. Journal of Neurochemistry, 2007. 101(5): p. 1367-1379.
6. Danno, S., et al., Increased transcript level of RBM3, a member of the glycine-rich RNA-binding protein family, in human cells in response to cold stress. Biochemical and Biophysical Research Communications, 1997. 236(3): p. 804-807.
7. Chip, S., et al., The RNA-binding protein RBM3 is involved in hypothermia induced neuroprotection. Neurobiology of Disease, 2011. 43(2): p. 388-396.
8. Peretti, D., et al., RBM3 mediates structural plasticity and protective effects of cooling in neurode-generation. Nature, 2015. 518: p.236-239
9. Zeng, Y., Stress-response protein RBM3 attenuates the stem-like properties of prostate cancer cells by interfering with CD44 variant splicing. 2013.73 (13): p.4123-33
10. Olofsson, S., et al., Low RBM3 protein expression correlates with clinical stage, prognostic classification and increased risk of treatment failure in testicular non-seminomatous germ cell cancer. PLoSOne. 2015. 10 (3): e0121300

12. Ehlen, A., et al., Expression of the RNA-binding protein RBM3 is associated with a favourable prognosis and cisplatin sensitivity in epithelial ovarian cancer. J. Trans. Med., 2010, 8:78
13. Boman, K., Decreased expression of RNA-binding motif protein 3 correlates with tumour progression and poor prognosis in urothelial bladder cancer. BMC Urol. 2013. 8: 13-17
14. Nodin, B., et al., High MCM3 expression is an independent biomarker of poor prognosis and correlates with reduced RBM3 expression in a prospective cohort of malignant melanoma. Diagn. Pathol. 2012 (7): 82



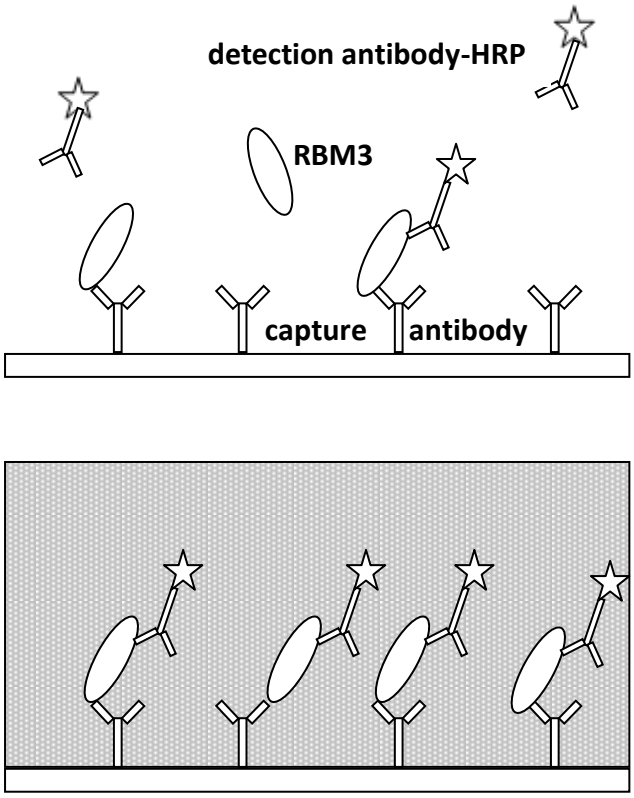
Intended use

The Human RBM3 ELISA provides a fast, highly sensitive and specific quantitative of RNA-binding protein 3 (RBM3) in human serum or plasma samples. Do not use for any other biological sample. One kit contains reagents for 96 determinations, thus allowing the measurement of one standard curve and 40 samples in duplicate.
The calibration curve covers the range from 31.25 pg/ml to 2000 pg/ml. The sensitivity of the assay is 10 pg/ml. The dilution of the serum or plasma samples is recommended 1:10.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Test principle

The Human RBM3 ELISA (Enzyme-Linked Immunosorbent Assay) is an immunological quantitative detection method based on a sandwich ELISA.
Human RBM3 ELISA is performed in a 96-well microplate. The wells of the microplate are pre-coated with a specific capture antibody against human RBM3 that binds free RBM3 from the patient sample. In one step simultaneous incubation of the RBM3 standards or samples and of the detection conjugate (peroxidase conjugate of an anti-RBM3 antibody) is carried out. Incubation takes place 2 hours at 20-25°C. After a washing step, the colour substrate TMB (tetramethylbenzidine) is added to the wells. The reaction is stopped by adding the Stop Solution and the yellow colour is read in a microtiter plate reader at 450 nm. The concentration of RBM3 in a sample is determined by interpolation from the standard curve.



Step 1: Incubation of RBM3 standard or sample and HRP labelled detection antibody on the microtiter plate. Specific binding of RBM3 and detection antibody (duration: 120 minutes)

Step 2: Washing step
(duration: about 5 minutes)

Step 3: Colour development after addition of TMB Substrate
(duration: 30 minutes)

Fig. 1: Scheme of the assay procedure

Safety warning and precautions

Warning: The kit contains substances such as 1M sulphuric acid, ProClin 300 (maximum 0.04%) as a preservative in reagents and TMB that are corrosive and/or toxic. Avoid contact with eyes and skin! Wear protective gloves!
The Standard Diluent contains material of animal origin that should be regarded as potentially infectious. You should therefore observe the appropriate protection regulations concerning the handling and disposal of these materials. In the event of injury a medical specialist should always be consulted.
All chemicals should be considered as being potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and

that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing, such as laboratory overalls, safety glasses and gloves. Avoid contact with skin and eyes. In case of skin and eyes contact, wash immediately with water.

Storage

All components of the kit should be stored in the refrigerator (2-8 °C).
Once reconstituted, the **RBM3-Standard-CONC** should be used immediately or stored at -20 °C.
Diluted **RBM3-CONJ** should be prepared freshly directly before use. When running a partial plate, only suitable aliquots of these solutions should be made. After dilution **WASHBUF** is stable for 4 weeks at storage temperature 2-8°C.

Components of the assay system

No.	Components	Marking	Volume	Colour of top cap	Con-dition **
1	Microplate coated with anti-RBM3 antibody	**RBM3-PLATE**	12 Strips á 8 well	-	G
2	Sample dilution buffer	**RBM3-SAMPLE-BUF**	1 x 25.0 mL	colourless	G
3	RBM3 Standard concentrate (12 ng RBM3 lyophilized)	**RBM3-STANDARD-CONC**	1 x	yellow	R
4	RBM3 dilution buffer	**RBM3-DILUTION-BUF**	1 x 10.0 mL	blue	G
5	RBM3 conjugate concentrate	**RBM3-CONJ-CONC**	1 x 0.1 mL	black	R
6	RBM3 conjugate dilution buffer	**RBM3-CONJ-BUF**	1 x 8.0 mL	green	G
7	TMB reagent	**TMB**	1 x 12.0 mL	brown	G
16	Stopping solution (1M H_2SO_4)	**STOP-H_2SO_4**	1 x 4.0 mL	colourless	G
17	Washing buffer concentrate (10x)	**WASHBUF 10x**	1 x 30.0 mL	colourless	R
18	Cover sheeting for microplate	**-**	1 piece	-	G

*** Condition: G = ready for use; R = reconstitution required**

RBM3-PLATE: The plate contains 12 strips (96 wells) coated with monoclonal anti-RBM3 antibody. Ready to use.
RBM3-SAMPLE-BUF: Bottle contains 25 ml buffer containing additives. Ready to use.
RBM3-STANDARD-CONC (lyophilised): The vial contains 12 ng of lyophilized RBM3. It has to be reconstituted with 1 ml of destilled water prior to use to get a 12 ng/ml standard solution.
RBM3-DILUTION-BUF: Solution to produce RBM3-STANDARDS after reconstitution RBM3-STANDARD-CONC (lyophilized). Ready to use.

RBM3-CONJ-CONC: Solution (100 µl) contains the detection antibody (HRP-labelled monoclonal anti-RBM3 antibody) in storage buffer with additives. The antibody has to be diluted 200-fold with RBM3-CONJ-BUF before use.
RBM3-CONJ-BUF: Solution (8 ml) consists of buffer containing additives. Ready to use.
WASHBUF 10x: Bottle contains 30 ml buffer concentrate. It has to be diluted 10-fold with distilled or deionised water before use.
TMB: Bottle contains 12 ml of a TMB solution ready to use.
STOP-H_2SO_4: Bottle contains 4 ml 1 M H_2SO_4. Ready to use. **Warning**: Stop solution contains 1 M sulfuric acid. Wear eye, hand, face and clothing protection when using this material!

EQUIPMENTS REQUIRED BUT NOT PROVIDED

- Pipettes with disposable tips (50 µl, 100 µl and 1 ml), a multi-channel pipette (100 µl) would be appropriate
- Distilled or deionised water
- Horizontal orbital microplate shaker
- Microplate reader capable of measuring at 450 nm

Sample preparation and storage

Serum:

- Serum or plasma samples (EDTA or Heparin as anticoagulant) may be stored at -20°C or -80°C. When stored at -20°C or -80°C, it is absolutely necessary to mix the samples thoroughly prior to measuring. Avoid freeze-thaw cycles.
- Dilute the serum or plasma samples minimum **1:10** with RBM3-SAMPLE-BUF, depending on the possible concentration of the analyte.

Critical Parameters

- Allow samples and all reagents to equilibrate to room temperature (20-25°C) prior to performing the assay. This is especially a prerequisite for reconstituted WASHBUF and TMB!
- It is absolutely important that all wells are washed thoroughly and uniformly. When washing is done by hand, ensure that all wells are completely filled and emptied at each step by discarding the contain of the plate with forceful motion and drying by tapping the inverted plate on dry absorbent surface (see technical advice on the following link: http://www.youtube.com/watch?v=FZirnCas17Y)
- Use only reagents from the same lot for each assay. This is especially important when running more than one plate per sample group.
- A separate standard curve must be run on each plate.
- Mix all reagents thoroughly prior to use, but **avoid foaming**!
- Keep the wells sealed with the foil except when adding reagents and during reading.
- Any variation in the protocol can cause variation in binding!
- The kit should not be used beyond the expiration date on the kit label.
- The values obtained by the samples should be within the standard range. If this is not the case, dilute the sample and repeat the assay.

- We take great care to ensure that this product is suitable for all validated sample types, as designated in this manual. However, it is possible that in some cases, high levels of interfering substances may cause unusual results.

Preparation of reagents

Please note: To prevent margin effects it is absolutely necessary to equilibrate all reagents **to room temperature** prior to use. For the dilution of the WASHBUF 10x use either distilled or deionised water. Always seal the plates with the provided foil during incubation!

RBM3-PLATE: After equilibration to room temperature the plate is ready to use. The plate should be removed from its packaging until immediately before start of pipetting.
RBM3-SAMPLE-BUF: After equilibration to room temperature the buffer is ready to use.
RBM3-STANDARD-CONC (lyophilised): Add 1 ml destilled water to the standard tube (yellow lid) and allow the contents to dissolve for 5-10 minutes. Gently mix, but avoid foaming of the reagent!
RBM3-DILUTION-BUF: After equilibration to room temperature the reagent is ready to use.
RBM3-CONJ-CONC: After equilibration to room temperature dilute this reagent 200-fold with RBM3-CONJ-BUF. For a whole plate add 30 µl from the RBM3-CONJ-CONC (black lid) to 6 ml RBM3-CONJ-BUF. When running half a plate, add 15 µl RBM3-CONJ-CONC solutions to 3 ml RBM3-CONJ-BUF.
RBM3-CONJ-BUF: After equilibration to room temperature the reagent is ready to use.
WASHBUF 10x: After equilibration to room temperature dilute the WASHBUF 10x concentrate 1:10 with distilled or deionised water (for example: 20 ml WASCHBUF 10x to 200 ml).
TMB and STOP-H_2SO_4: After equilibration to room temperature the reagents are ready to use.

Preparation of RBM3 standards with RBM3-DILUTION-BUF

1. Label 7 tubes with 31.25, 62.5, 125, 250, 500, 1000 and 2000 pg/ml.
2. Pipette 500 µl of RBM3-DILUTION-BUF into the 2000 pg/ml tube, in the remaining tubes pipette 300 µl of RBM3-DILUTION-BUF.
3. Pipette 100 µl of the RBM3-STANDARD-CONC (12 ng/ml) into the 2000 pg/ml tube and mix thoroughly.
4. Pipette 300 µl of the 2000 pg/ml standard into the tube labelled with 1000 pg/ml and mix thoroughly.
5. Repeat this dilution procedure with the other standard tubes.
6. The blank value (0 pg/ml) is obtained by using only RBM3-DILUTION-BUF.
7. The stock solution is not part of the standard curve and can be stored at -20°C.

Assay protocol

1. Prepare reagents and standards as described in the sections above. **Remind that it is necessary to equilibrate the reagents to room temperature before use**.
2. Prepare the unknown samples as described above by appropriate dilution with RBM3-SAMPLE-BUF. Recommended Dilution of the serum or plasma samples 1:10 (for example: 30 µl sample to 270 µl RBM3-SAMPLE-BUF)
3. Prepare the Microtiter plate by inserting the required amount of wells into the frame. **Note that you need 16 wells for the standard curve**.

4. **Standard curve:** Pipette 50 µl of the reconstituted standards 31.25, 62.5, 125, 250, 500, 1000 and 2000 pg/ml in duplicate in the wells using a clean pipette tip for each standard. RBM3-Dilution-BUF serves as zero blank.
5. **Samples :** Pipette 50 µl of the prepared unknown samples in duplicate into the wells.
6. Add 50 µl of diluted RBM3-CONJ into each well.
7. Seal the plate with the provided foil and incubate 5 min on a shaker at room temperature (20-25°C) and after that exactly 115 minutes at room temperature without shaking. Keep the plate in the dark.
8. Wash by filling each well with diluted WASHBUF (300 µl), then remove by discarding/drying by tapping inverted plate against clean paper towels. Take care that all wells are completely filled and emptied at each wash. Wash the wells 3 times with diluted WASHBUF.
9. Add 100 µl of TMB solution to each well.
10. Seal the plate with foil provided and incubate in the dark at room temperature on a shaker for 30 minutes.
11. Stop the reaction by adding 25 µl of STOP-H_2SO_4 to each well.
12. Read the plate at 450 nm (620 nm reference filter). Reading of the plate without reference may yield higher absorbance and thus may be less accurate.

Protocol summary

Prepare reagents, standards and samples as described, equilibrate reagents to **room temperature.**

↓

Pipette 50 µl standard or sample in duplicate into the wells. Pipette 50 µl diluted RBM3-CONJ to each well. Incubate 5 min on a shaker, than incubate 115 minutes at room temperature in the dark.

↓

Discard/Dry by tapping/Wash 3 times

↓

Add 100 µl of TMB substrate to each well. Incubate for 30 minutes at room temperature in the dark on a shaker!

↓

Add 25 µl STOP-H_2SO_4 solution to each well. Read at 450 nm (with reference filter at 620 nm).

Scheme of the plate

Strip	1	2	3	4	5	6
Row	Field of RBM3 calibrators		Field of diluted samples			
A	RBM3-Standard 0 pg/ml	RBM3-Standard 0 pg/ml	Sample 1	Sample 1	Sample 9	Sample 9
B	RBM3-Standard 31.25 pg/ml	RBM3-Standard 31.25 pg/ml	Sample 2	Sample 2	Sample 10	Sample 10
C	RBM3-Standard 62.5 pg/ml	RBM3-Standard 62.5 pg/ml	Sample 3	Sample 3	Sample 11	Sample 11
D	RBM3-Standard 125 pg/ml	RBM3-Standard 125 pg/ml	Sample 4	Sample 4	Sample 12	Sample 12
E	RBM3-Standard 250 pg/ml	RBM3-Standard 250 pg/ml	Sample 5	Sample 5	Sample 13	Sample 13
F	RBM3-Standard 500 pg/ml	RBM3-Standard 500 pg/ml	Sample 6	Sample 6	Sample 14	Sample 14
G	RBM3-Standard 1000 pg/ml	RBM3-Standard 1000 pg/ml	Sample 7	Sample 7	Sample 15	Sample 15
H	RBM3-Standard 2000 pg/ml	RBM3-Standard 2000 pg/ml	Sample 8	Sample 8	Sample 16	Sample 16

Example of the layout for pipetting RBM3 standards and samples on the plate

Data processing

Calculation of results

The calculation is illustrated using representative data: the assay data should be similar to that shown in table 1.

1. Calculate the average absorbance for each set of standard wells.
2. A standard curve is generated by plotting the mean absorbance (x-axis, fig. 2) against pg/ml standard (y-axis, fig.2).
3. The pg/ml values of the samples can be read directly from the graph or calculated by the regression coefficients.
4. Multiply the calculated pg/ml values by the dilution factor of the samples.

RBM3 Standard (pg/ml)	RBM3 Standard curve Absorbance (450 nm)		
	Value 1	Value 2	Mean Value
0.00	0.0130	0.0110	0.0120
31.25	0.0350	0.0370	0.0360
62.50	0,0650	0.0710	0.0680
125.00	0.1420	0.1480	0.1450
250.00	0.3410	0.3470	0.3440
500.00	0.7250	0.7670	0.7460
1000.00	1.5640	1.5240	1.5440
2000.00	2.5610	2.5630	2.5620

Table 1: Typical assay data

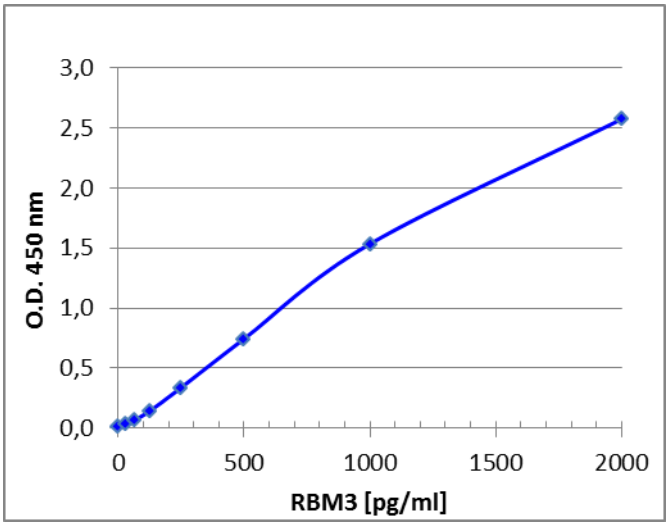


Fig. 2: Typical RBM3 standard curve

Additional Information

Specificity

The RBM3 ELISA has a high sensitivity and high specificity for quantitative determination of human RBM3. It does not cross-react with human Cold inducible Binding protein (CIRBP).

Sensitivity

The minimum detectable dose of human RBM3 is less than 10 pg /ml. The lower limit of detection was defined as the lowest protein concentration that could be differentiated from zero. It was determined the mean O.D. value of 20 replicates of the zero standard added by their three standard deviations.

Linearity

The assay should be conducted with a serum dilution of 1:10. For dilutions of 1:20 to 1:30, the linearity of the assay results to their dilution is assured.

Recovery

The recovery of RBM3 standard spiked to levels throughout the range of the assay in serum.

Matrix	Recovery range (%)
Serum (n=6)	82-110%

Reproducibility

Within assay precision

The within assay precision was measured by assaying 3 serum samples with different levels of the antigen RBM3 on 1 plate, 20 replicates of each sample.

Sample	Mean value RBM3 [pg/mL]	CV (%)	N
1.	1711	1.9	20
2.	5521	2.3	20
3.	10407	1.7	20

Between assay precision

The between assay variation was measured by assaying 3 serum samples with different levels of the antigen RBM3 on 3 plates, 20 replicates in each plate.

Sample	Mean value RBM3 [pg/mL]	CV (%)	N
1.	1712	2.6	60
2.	5576	2.7	60
3.	10521	3.0	60

Troubleshooting

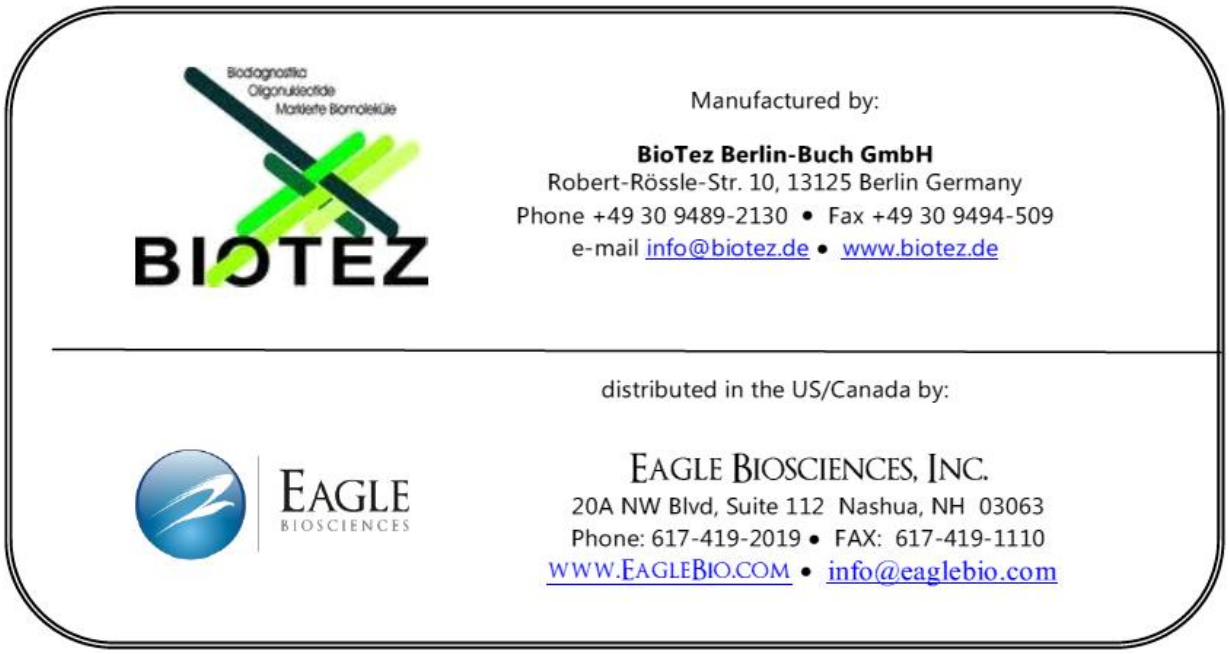
Problem	Potential cause	Recommendation
Low absorbance	• Wrong wavelength • Enzyme conjugate out of date/reagents improperly stored • Improper incubation time and temperature • Reagents not equilibrated to RT • Reagents not correctly prepared	• Check reader wavelength • Control the expiration date/storage conditions • Control the incubation time and temperature • Check equilibration of reagents to RT • Check preparation of reagents
High absorbance/ high zero standard value	• Incomplete washing • Improper removing of residual fluid • Improper incubation time and temperature • Reagents not equilibrated to RT • Reagents not correctly prepared	• Ensure that every well is completely filled/emptied during each washing step • Check that plates are blotted on tissue paper after each washing step • Control the incubation time and temperature • Check equilibration of reagents to RT • Check preparation of reagents
Flat curve/poor reproducibility	• Wrong wavelength • Enzyme conjugate out of date/reagents improperly stored • Improper preparation of working standards • Pipette errors • Contamination of components by use of unclean reservoirs/used pipette tips • Margin effects by using of cold substrate solution • Washing incomplete	• Check wavelength • Control the expiration date/storage conditions • Check preparation of standards • Check pipette calibration • Use separate reservoirs and always new pipette tips • Equilibrate substrate to room temperature • Ensure sufficient washing procedure

Related Products

For detection of human RBM3 you can use our antibody:

Cat.No.: 12 100 001 monoclonal antibody RBM3, human

More antibodies information you will find in our catalogue: see to http://www.biotez.de/



Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.