MANUAL

HUMAN MBNL1 KITS

Part # 63ADK012PEG & 63ADK012PEH

Test size#: 500 tests (63ADK012PEG) and 10,000 tests (63ADK012PEH) - assay volume: 20 µL

Revision: #06 of March 2024

Store at: -16°C or below (63ADK012PEG); -16°C or below (63ADK012PEH)

For research use only. Not for use in diagnostic procedures.

ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of human muscleblind-like protein-1(MBNL1) in cell lysates and offers a fast alternative to ELISA.

The detection principle of this kit is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). As shown in Figure 1, MBNL1 is detected in a sandwich assay by using an anti MBNL1 antibody labeled with Europium cryptate (donor), and an anti MBNL1 antibody labeled with d2 (acceptor).

When the dyes are in close proximity, the excitation of the donor with a light source (laser or flash lamp) triggers a Fluorescence Resonance Energy Transfer (FRET) towards the acceptor, which in turn fluoresces at a specific wavelength (665 nm). Signal intensity is proportional to the number of antigen-antibody complexes formed and therefore to the MBNL1 concentration.

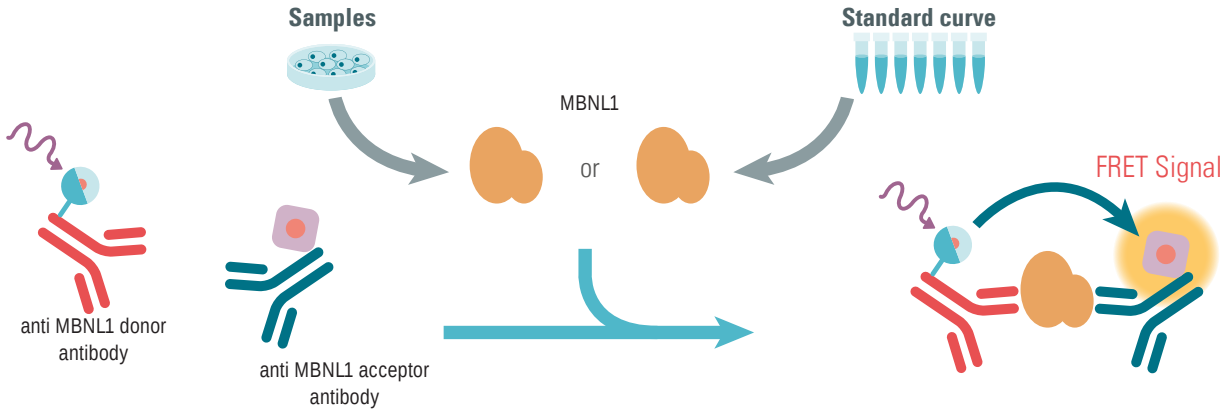
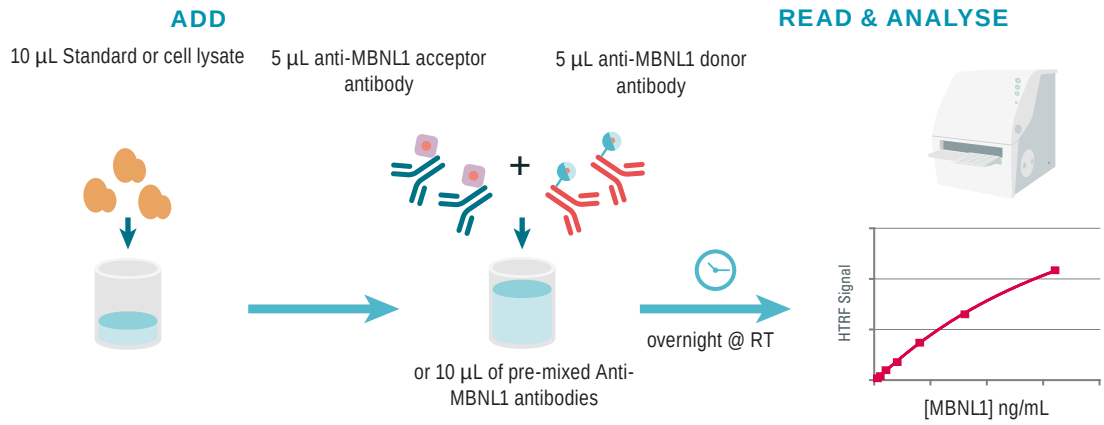


Figure 1: Principle of HTRF MBNL1 sandwich assay.

MANUAL AT A GLANCE



Make sure to use the set-up for Eu Cryptate.

MATERIALS PROVIDED:

KIT COMPONENTS	500 TESTS * CAT # 63ADK012PEG	10,000 TESTS * CAT # 63ADK012PEH
MBNL1 Standard Frozen	1 vial - 10 µL 100 µg/mL	1 vial - 10 µL 100 µg/mL
MBNL1 Eu Cryptate Antibody	1 vial - 50 µL Frozen - 50X	1 vial - 1 mL Frozen - 50X
MBNL1 d2 Antibody	1 vial - 50 µL Frozen - 50X	1 vial - 1 mL Frozen - 50X
Lysis buffer ** 4X	1 vial 2 mL	1 vial 130 mL
Detection buffer *** ready-to-use	1 vial 7 mL Detection Buffer #3	1 vial 105 mL Detection Buffer #3

* When used as advised, the two available kit sizes will provide sufficient reagents for 500 tests and 10,000 tests respectively in 20 µL final volume. Assay volumes can be adjusted proportionally to run the assay in 96 or 1536 well microplates.
** Medium like cell culture medium can be an alternative to the diluent.
*** The Detection buffer is used to prepare working solutions of acceptor and donor reagents.

PURCHASE SEPARATELY:

- HTRF®-Certified Reader. Make sure the setup for Eu Cryptate is used.

For a list of HTRF-compatible readers and set-up recommendations, please visit www.revvity.com

- Small volume (SV) detection microplates - .

For more information about microplate recommendations, please visit our website at: www.revvity.com

STORAGE AND STABILITY

Store the kit at -16°C or below.

Under proper storage conditions, reagents are stable until the expiry date indicated on the label. Detection buffer and lysis buffer are shipped frozen, but can be stored at 2-8°C in your premises.

If lyophilized, reconstituted reagents, antibodies, and standard stock solutions may be frozen and thawed only once. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.

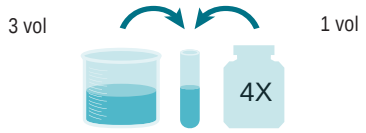
REAGENT PREPARATION

BEFORE YOU BEGIN:

- It is very important to prepare reagents in the specified buffers. The use of an incorrect lysis buffer may affect reagent stability and assay results.
- Thaw the frozen reagents at room temperature, allow them to warm up to room temperature for at least 30 mins before use
- Before use, allow Lysis buffer and Detection buffer to warm up at room temperature and homogenize them with a vortex.
- Antibody solutions must be prepared in individual vials and can be mixed prior to dispensing.
- MBNL1 standards (for standard curve) must be prepared in lysis buffer or in the same medium as the samples.

TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

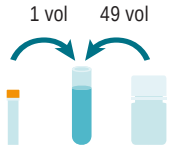
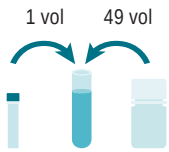
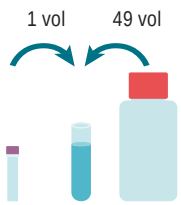
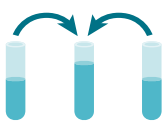
TO PREPARE REAGENT STOCK SOLUTIONS:

500 TESTS KIT - 63ADK012PEG		10,000 TESTS KIT - 63ADK012PEH
Anti-MBNL1 Eu Cryptate antibody		
Thaw the MBNL1 Eu Cryptate antibody . Mix gently. This 50X stock solution can be frozen and stored at -16°C or below.		Thaw the MBNL1 Eu Cryptate antibody . Mix gently. This 50X stock solution can be frozen and stored at -16°C or below.
Anti-MBNL1 d2 antibody		
Thaw the MBNL1 d2 antibody . Mix gently. This 50X stock solution can be frozen and stored at -16°C or below.		Thaw the MBNL1 d2 antibody . Mix gently. This 50X stock solution can be frozen and stored at -16°C or below.
MBNL1 Standard		
Thaw the MBNL1 Standard in order to obtain a 100 µg/mL stock solution. Mix gently. This stock solution can be frozen and stored at -20°C or below.		Thaw the MBNL1 Standard in order to obtain a 100 µg/ mL stock solution. Mix gently. This stock solution can be frozen and stored at -20°C or below.
Lysis buffer		
Prepare only the amount of lysis buffer needed for the experiment. Dilute 4-fold the 4 X lysis buffer with distilled water: homogenize the 4 X lysis buffer with a vortex and add 1 volume of stock solution in 3 volumes of distilled water (e.g., 1.25 mL of lysis buffer + 3.75 mL of distilled water). Mix gently after dilution. This 1 X lysis buffer is stable for 2 days at 2-8°C.		Prepare only the amount of lysis buffer needed for the experiment. Dilute 4-fold the 4 X lysis buffer with distilled water: homogenize the 4 X lysis buffer with a vortex and add 1 volume of stock solution in 3 volumes of distilled water (e.g., 1.25 mL of lysis buffer + 3.75 mL of distilled water). Mix gently after dilution. This 1 X lysis buffer is stable for 2 days at 2-8°C.
Detection buffer		
The Detection buffer is ready-to-use.		The Detection buffer is ready-to-use.

TO PREPARE ANTIBODY WORKING SOLUTIONS:

Each well requires 5 µL of MBNL1-Eu Cryptate Antibody and 5 µL of MBNL1-d2 Antibody.

Prepare the two antibody solutions in separate vials.

500 TESTS KIT - 63ADK012PEG		10,000 TESTS KIT - 63ADK012PEH
MBNL1 Eu Cryptate antibody		
Dilute 50-fold the 50X stock solution (thawed reagent) of MBNL1 Eu Cryptate antibody with the Detection buffer #3: add 1 volume of Eu Cryptate antibody stock solution in 49 volumes of Detection buffer #3 (e.g., 0.05mL of reconstituted Eu Cryptate antibody stock solution + 2.45 mL of Detection buffer #3).	 	Dilute 50-fold the 50X stock solution (thawed reagent) of MBNL1 Eu Cryptate antibody with the Detection buffer #3: add 1 volume of Eu Cryptate antibody stock solution in 49 volumes of Detection buffer #3 (e.g. take 1 mL of Eu Cryptate antibody stock solution and add it to 49 mL of Detection buffer #3)
MBNL1 d2 antibody		
Dilute 50-fold the 50X stock solution (thawed reagent) of MBNL1 d2 antibody with the Detection buffer #3: add 1 volume of d2 antibody stock solution in 49 volumes of Detection buffer #3 (e.g., 0.05 mL of reconstituted d2 antibody stock solution + 2.45 mL of Detection buffer #3).	 	Dilute 50-fold the 50X stock solution (thawed reagent) of MBNL1 d2 antibody with the Detection buffer #3: add 1 volume of d2 antibody stock solution in 49 volumes of Detection buffer #3 (e.g. take 1 mL of d2 antibody stock solution and add it to 49 mL of Detection buffer #3)
Antibody mix		
It is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of d2 antibody solution to 1 volume of Cryptate antibody solution (e.g. 1 mL of d2 antibody + 1 mL of Cryptate antibody).		It is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of d2 antibody solution to 1 volume of Cryptate antibody solution (e.g. 1 mL of d2 antibody + 1 mL of Cryptate antibody).

TO PREPARE STANDARD WORKING SOLUTIONS:

- Each well requires 10 µL of standard.
- Dilute the standard stock solution serially with lysis buffer (1X)
- In order to check for a potential interference effect from your own assay buffer when using the assay for the first time, we highly recommend the parallel preparation of a standard curve in your own supplemented cell culture medium and in lysis buffer (1X).
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

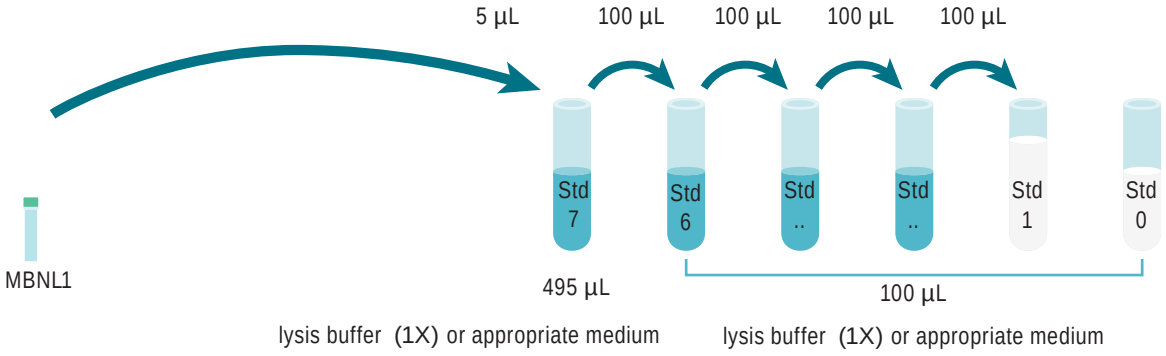
A recommended standard dilution procedure is listed and illustrated below:

Dilute the standard stock solution 100-fold with lysis buffer (1X) to prepare high standard (Std 7): e.g. take 5 µL of standard stock solution and add it to 495 µL of lysis buffer (1X). Mix gently.

Use the high standard (Std 7) to prepare the standard curve using 1/2 serial dilutions as follows:

- Dispense 100 µL of lysis buffer (1X) in each vial from Std 6 to Std 0.
- Add 100 µL of standard to 100 µL of lysis buffer (1X), mix gently and repeat the 1/2 serial dilution to make standard solutions: std6, std5, std4, std3, std2, std1.

This will create 7 standards for the analyte. Std 0 (Negative control) is lysis buffer (1X) or appropriate culture medium alone.



STANDARD	SERIAL DILUTIONS	HUMAN MBNL1 WORKING SOLUTIONS (ng/mL)
Standard Stock solution	Thawed stock solution	100,000
Standard 7	5 µL Standard stock solution + 495 µL lysis buffer 1X	1,000
Standard 6	100 µL standard 7 + 100 µL lysis buffer 1X	500
Standard 5	100 µL standard 6 + 100 µL lysis buffer 1X	250
Standard 4	100 µL standard 5 + 100 µL lysis buffer 1X	125
Standard 3	100 µL standard 4 + 100 µL lysis buffer 1X	62.5
Standard 2	100 µL standard 3 + 100 µL lysis buffer 1X	31.25
Standard 1	100 µL standard 2 + 100 µL lysis buffer 1X	15.62
Standard 0	100 µL lysis buffer	0

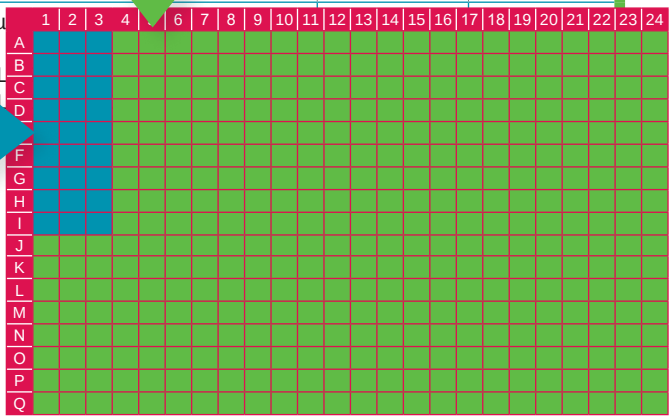
TO PREPARE SAMPLES:

- Each well requires 10 µL of sample.
- Just after their collection, put the samples at 4°C and test them immediately. For later use, samples should be dispensed into disposable plastic vials and stored at -60°C or below. Avoid multiple freeze/thaw cycles.
- Samples with a concentration above the highest standard (Std 7) must be diluted lysis buffer (1X)
- The assay can be run under a two-plate manual, where cells are plated and stimulated in the same culture plate, then transferred to the assay plate for the HTRF® detection. This manual enables the cells' viability and confluence to be monitored. It can also be further streamlined to a one-plate assay manual where plating, stimulation and detection is performed in a single plate. For two-plate & one- plate assay manuals for suspension cells and adherent cells kept in medium for the lysis, we recommend to use the lysis buffer 4X (ready to use) For two-plate & one-plate assay manuals for adherent cells removing the medium for lysis, we recommend to use the lysis buffer 1X. We recommend to incubate the cells with lysis buffer for at least 30 minutes at room temperature under shaking. Depending on cell lines used, it can be necessary to extend the lysis step up to 1 hour. Other parameters such as cell density and stimulation time are cell-dependent and need to be optimized.
- To obtain additional information or support, please contact the HTRF technical support team at www.revvity.com

ASSAY MANUAL

	Standard (Std 0 - Std 7)	Samples
Step 1	Dispense 10 µL of each MBNL1 standard (Std 0 - Std 7) into each standard well	Dispense 10 µL of each sample into each sample well
Step 2	Add 5 µL of MBNL1 d2 antibody working solution to all wells	
Step 3	Add 5 µL of MBNL1 Eu Cryptate antibody working solution to all wells	
Step 4	Seal the plate and incubate overnight @ RT	
Step 5	Remove the plate sealer and read on an HTRF® compatible reader	

	1	2	3	4	5	6
A	10 µL Std 0 (Negative control) 5 µL MBNL1-d2 5 µL MBNL1-Eu Cryptate	Repeat Well A1	Repeat Well A1	10 µL Sample 1 5 µL MBNL1-d2 5 µL MBNL1-Eu Cryptate	Repeat Well A4	Repeat Well A4
B	10 µL Std 1 5 µL MBNL1-d2 5 µL MBNL1-Eu Cryptate	Repeat Well B1	Repeat Well B1	10 µL Sample 2 5 µL MBNL1-d2 5 µL MBNL1-Eu Cryptate	Repeat Well B4	Repeat Well B4
C	10 µL Std 2 5 µL MBNL1-d2 5 µL MBNL1-Eu Cryptate	Repeat Well C1	Repeat Well C1	10 µL Sample 3 5 µL MBNL1-d2 5 µL MBNL1-Eu Cryptate	Repeat Well C4	Repeat Well C4
D	10 µL Std ... 5 µL MBNL1-d2 5 µL MBNL1-Eu Cryptate	Repeat Well D1	Repeat Well D1	10 µL Sample ... 5 µL MBNL1-d2 5 µL MBNL1-Eu Cryptate	Repeat Well D4	Repeat Well D4
E	10 µLStd ... 5 µL MBNL1-d2 5 µL MBNL1-Eu Cryptate	Repeat Well E1	Repeat Well E1	10 µL Sample ... 5 µL MBNL1-d2 5 µL MBNL1-Eu Cryptate	Repeat Well E4	Repeat Well E4
F	10 µL Std ... 5 µL MBNL1-d2 5 µL MBNL1-Eu Cryptate	Repeat Well F1	Repeat Well F1	10 µL Sample ... 5 µL MBNL1-d2 5 µL MBNL1-Eu Cryptate	Repeat Well F4	Repeat Well F4
G	10 µL Std ... 5 µL MBNL1-d2 5 µL MBNL1-Eu Cryptate	Repeat Well G1	Repeat Well G1	10 µL Sample ... 5 µL MBNL1-d2 5 µL MBNL1-Eu C	Repeat Well G4	Repeat Well G4
H	10 µL Std ... 5 µL MBNL1-d2 5 µL MBNL1-Eu Cryptate	Repeat Well H1	Repeat Well H1	10 µ 5 µL		



DATA REDUCTION

1. Calculate the ratio of the acceptor and donor emission signals for each individual well.

$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$$

2. Calculate the % CVs. The mean and standard deviation can then be worked out from ratio replicates.

$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

3. Calculate the % delta F which reflects the signal to background of the assay. The negative control (Standard 0) plays the role of an internal assay control. Delta F is used for the comparison of day to day runs of the same assay.

$$\text{delta F (\%)} = \frac{\text{Ratio Standard or sample - Ratio Negative Control}}{\text{Ratio Negative Control}} \times 100$$

For more information about data reduction, please visit www.revvity.com

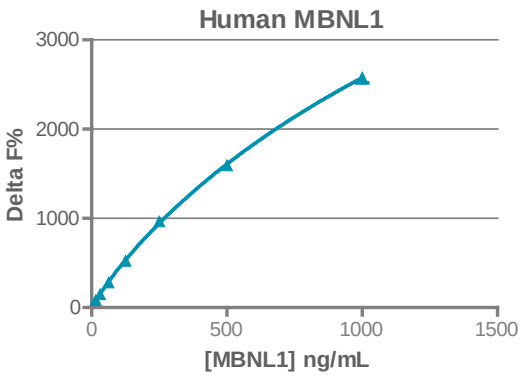
RESULTS

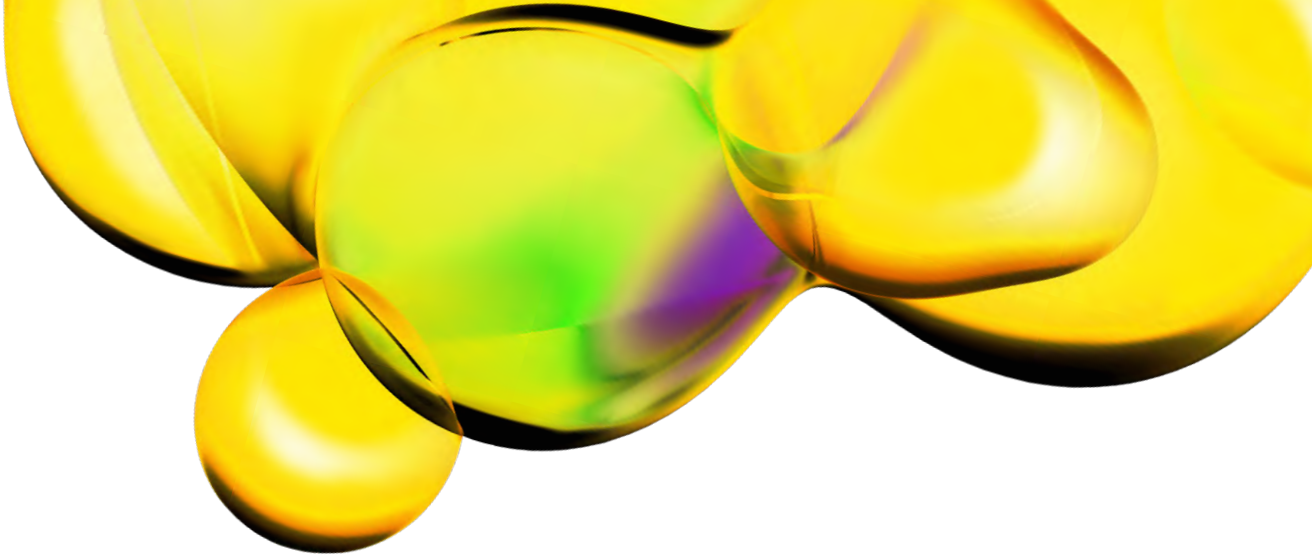
This data must not be substituted for the data obtained in the laboratory and should be considered only as an example.

Results may vary from one HTRF® compatible reader to another.

The assay standard curve is created by plotting delta F% versus the analyte concentration:

	Ratio (1)	CV (2)	Delta F% (3)
Standard 0 - Negative control	780	0%	2%
Standard 1 -15.6 ng/mL	1,403	2%	84%
Standard 2 - 31.25 ng/mL	1,890	9%	148%
Standard 3 - 62.5 ng/mL	2,902	3%	281%
Standard 4 - 125 ng/mL	4,744	3%	522%
Standard 5 - 250 npg/mL	8,126	4%	966%
Standard 6 - 500 ng/mL	12,917	5%	1,595%
Standard 7 - 1,000 ng/mL	20,413	4%	2,578%





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