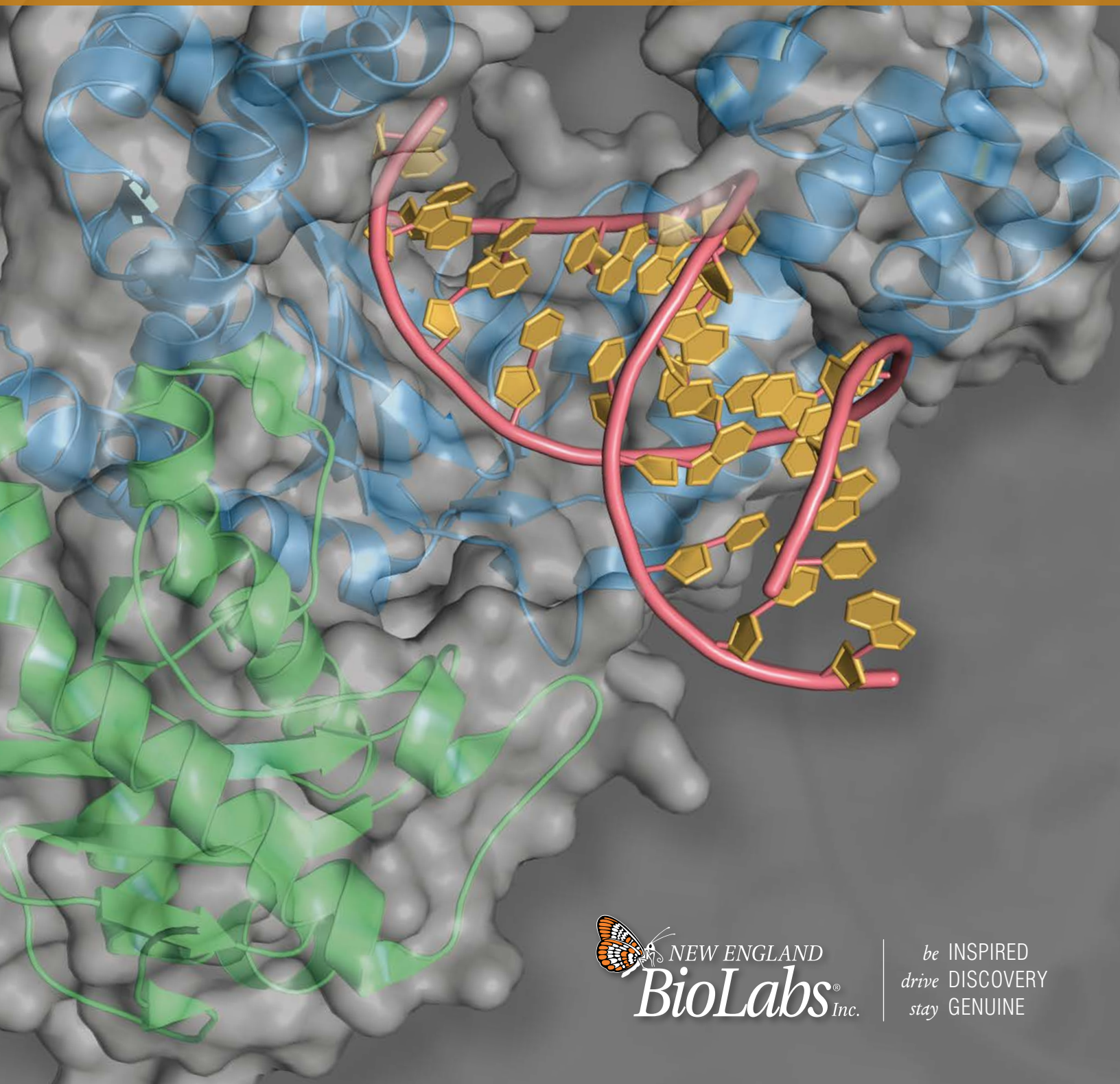
Isothermal Amplification

RAPID NUCLEIC ACID DETECTION FOR MOLECULAR DIAGNOSTICS



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What is isothermal DNA amplification?

The Polymerase Chain Reaction (PCR) is a well-known approach for amplifying a specific DNA sequence. PCR involves the reiterative cycling of a reaction cocktail between different temperatures to achieve amplification. As routine as PCR is in the molecular biology and molecular diagnostics laboratory, there are other methods of sequence-specific DNA amplification.

These alternative approaches often do not require changing the reaction temperature and are, therefore, often referred to as isothermal amplification protocols. Isothermal amplification protocols are varied and have different advantages. In general, isothermal techniques are extremely fast and do not require thermocyclers, making them particularly well suited for field applications and point-of-care molecular diagnostics assays.

Interested in learning how NEB scientists are using isothermal amplification?

Visit **www.neb.com/IsothermalAmplification** to find videos, protocols and recent publications, including a publication from NEB scientists describing pH-sensitive isothermal detection.

Advantages

- Fast
- Minimal equipment required
- Robust reactions in the presence of inhibitors
- Simplified optical detection

Optimization tips for LAMP

- Use LAMP primer design software (e.g., Primer Explorer – primerexplorer.jp/e/). Select 2–3 sets for each target and compare performance in a LAMP assay.
- Include loop primers for faster reactions
- Use high magnesium (6–8 mM) and dNTP (1–1.4 mM) concentrations for best reactions
- Omit betaine, unless it has a demonstrated benefit
- Optimize the reaction temperature (60–65°C for *Bst* LF and 63–70°C for *Bst* 2.0/3.0)
- To prevent contamination, use *Bst* 3.0 or Antarctic Thermolabile UDG (NEB #M0372), which denatures rapidly

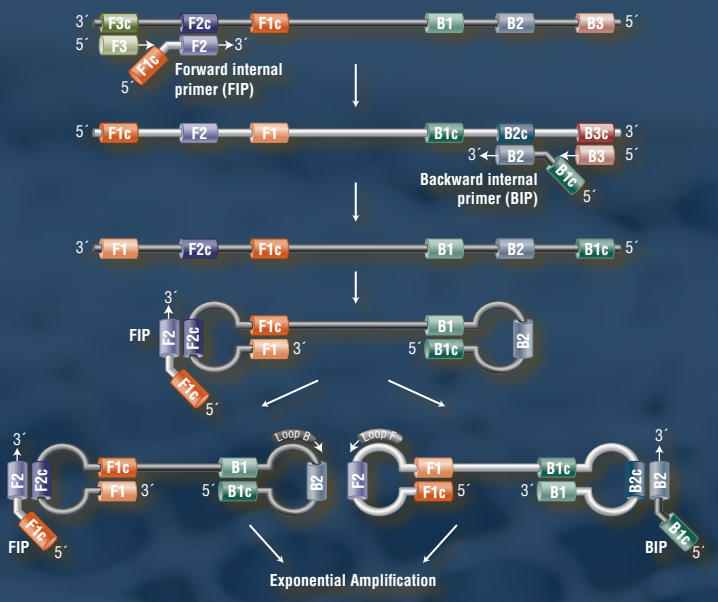
Examples of isothermal technologies

Loop-mediated Isothermal Amplification (LAMP & RT-LAMP)

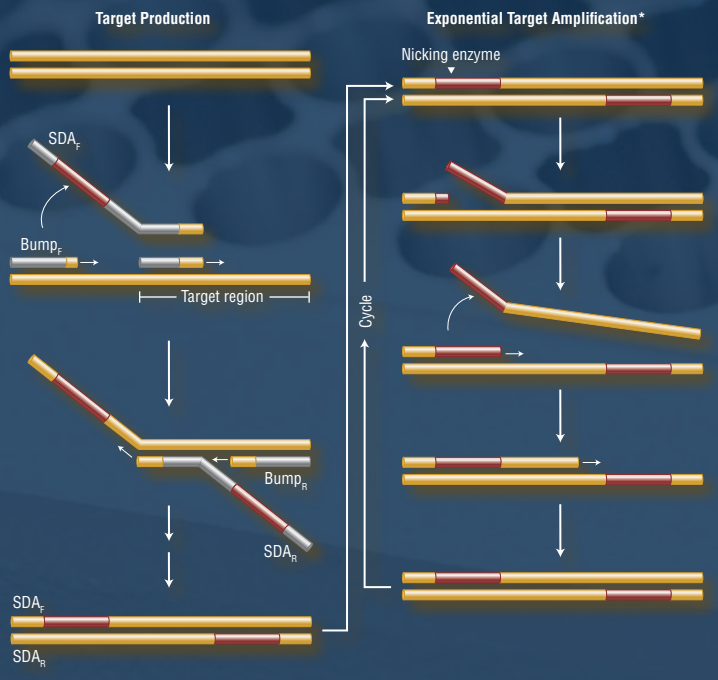
LAMP is designed to detect a target nucleic acid without sophisticated equipment. LAMP uses 4-6 primers recognizing 6-8 distinct regions of the target DNA. A strand-displacing DNA polymerase initiates synthesis and two of the primers form loop structures to facilitate subsequent rounds of amplification. LAMP provides high sensitivity (fg levels or <10 copies of target), and reactions can be performed in as little as 5–10 minutes. Additionally, reactions can be performed with limited resources (e.g., using a water bath for incubation, and detection of results by eye), or with real-time measurement and high-throughput instruments.

Detection of RNA targets is accomplished by simple addition of a reverse transcriptase to the LAMP reaction (e.g., WarmStart® RTx Reverse Transcriptase), with RT-LAMP performed as a true one-step, isothermal workflow.

Overview of LAMP



Overview of SDA



** Target amplification, shown above for SDA_F, will also occur simultaneously with SDA_R.*

Strand Displacement Amplification (SDA)

SDA relies on a strand-displacing DNA polymerase, typically *Bst* DNA Polymerase, Large Fragment (NEB #M0275) or Klenow Fragment (3´→ 5´exo⁻) (NEB # M0212), to initiate amplification at nicks created by a nicking enzyme (e.g., Nt.BstNBI, NEB # R0607) at a site contained in a primer. The nicking site is regenerated with each polymerase displacement step, resulting in exponential amplification. SDA is typically used in clinical diagnostics.

Helicase-dependent Amplification (HDA)

HDA employs the double-stranded DNA unwinding activity of a helicase to separate strands, enabling primer annealing and extension by a strand-displacing DNA polymerase. Like PCR, this system requires only two primers. HDA has been employed in several diagnostic devices and FDA-approved tests.

Nicking Enzyme Amplification Reaction (NEAR)

NEAR employs a strand-displacing DNA polymerase initiating amplification at a nick created by a nicking enzyme, rapidly producing many short nucleic acids from the target sequence. This process is extremely fast and sensitive, enabling detection of small target amounts in minutes. NEAR is commonly used for pathogen detection in clinical and biosafety applications.

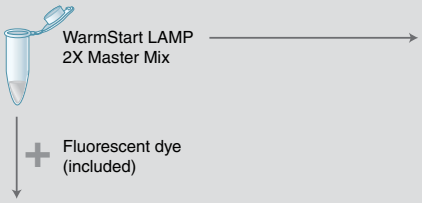
Featured Products for Isothermal

WarmStart LAMP KIT (DNA & RNA)

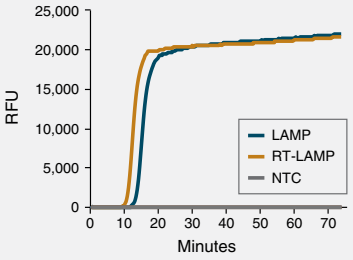
☑ Validated for LAMP & RT-LAMP

Loop Mediated Isothermal Amplification (LAMP) is a commonly-used technique for rapid nucleic acid detection. NEB's WarmStart LAMP products provide a simple, one-step solution for DNA or RNA targets. The master mix supplied with the WarmStart LAMP Kit contains the robust and rapid *Bst* 2.0 WarmStart DNA Polymerase and WarmStart RTx Reverse Transcriptase, both *in silico*-designed enzymes for improved performance in LAMP reactions. The kit also includes a fluorescent dye to enable real-time fluorescence measurement of LAMP. The WarmStart LAMP Kit is compatible with multiple detection methods.

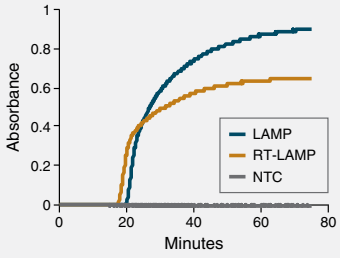
NEB's WarmStart LAMP Kit (DNA & RNA) is compatible with multiple detection methods*

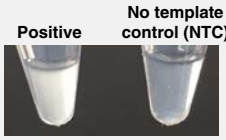


Real-time fluorescence measurement



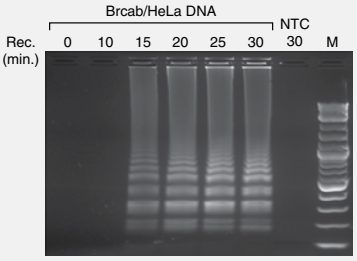
Turbidity detection





LAMP product is visible by eye due to the precipitation of magnesium pyrophosphate

Endpoint visualization

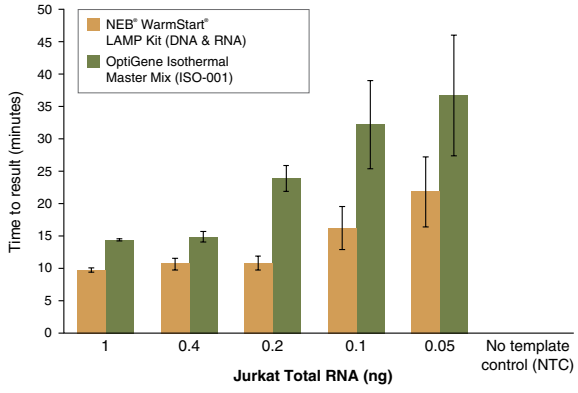


Agarose gel stained with EtBr

Visualization using a fluorescent intercalating dye (e.g., ethidium bromide, SYBR green or calcein/HNBlue)

** The NEB WarmStart LAMP Kit (DNA & RNA) includes separate fluorescent dye for real-time fluorescence measurement. Alternately, detection can be accomplished by turbidity detection or endpoint visualization.*

NEB's WarmStart LAMP Kit (DNA & RNA) offers speed and robust sensitivity



A RNA target (HMBS2) was amplified from Jurkat total RNA using the WarmStart LAMP Kit and OptiGene Master Mix (ISO-001). Reactions were performed at 65°C for 74 minutes on a real-time thermocycler (Bio-Rad CFX96) in triplicate. Time to result is set as the time at which the fluorescence crossed a threshold of 10% of maximal fluorescence. NEB's WarmStart LAMP Kit resulted in faster and more sensitive detection as compared to the OptiGene Master Mix.

WarmStart Colorimetric LAMP 2X Master Mix (DNA & RNA)

NEB's WarmStart Colorimetric LAMP 2X Master Mix (DNA & RNA) offers the same robust performance as the WarmStart LAMP Kit, but contains a colorimetric dye for best in class visual detection of your target.

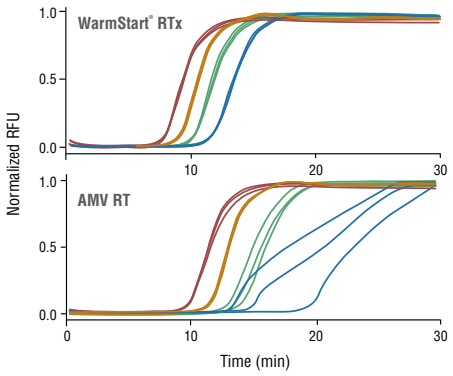
Amplification from NEB

WarmStart RTx Reverse Transcriptase

☑ Validated for RT-LAMP

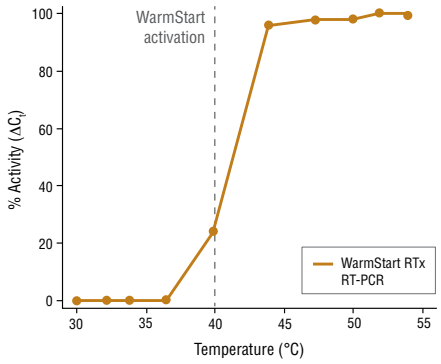
WarmStart RTx Reverse Transcriptase (NEB #M0380) is a unique *in silico*-designed, RNA-directed DNA polymerase coupled with a reversibly-bound aptamer that inhibits RTx activity below 40°C. This enzyme can synthesize a complementary DNA strand initiating from a primer using RNA (cDNA synthesis) or single-stranded DNA as a template. RTx is a robust enzyme for RNA detection in amplification reactions and is particularly well-suited for use in loop-mediated isothermal amplification (LAMP). The WarmStart property enables high throughput applications, room temperature setup, and increases the consistency and specificity of amplification reactions. RTx contains intact RNase H activity.

WarmStart improves speed and sensitivity in RT-LAMP



RT-LAMP reactions with Bst 2.0 WarmStart DNA Polymerase and the indicated reverse transcriptase were incubated at 65°C with 1 pg – 100 ng of Jurkat total RNA. Reactions were monitored with real-time fluorescence, and resulting curves are shown. WarmStart RTx provides faster reaction threshold times for improved consistancy and sensitivity with lower input RNA amounts. RT-LAMP reactions performed with AMV Reverse Transcriptase resulted in inconsistent detection, as indicated by wide variation at lower RNA input concentrations (blue curves).

WarmStart control of WarmStart RTx



cDNA synthesis was performed for 10 minutes, followed by qPCR analysis. Resulting Cts were normalized to a "no RT" control for 0% activity and fastest Ct for 100% activity. WarmStart RTx is inhibited by a reversibly bound aptamer at temperatures below 40°C, and is fully active at temperatures 42°C and higher.

Not sure which product will work best for your experiment?

NEB offers a selection of *Bst* DNA Polymerase-based products for isothermal DNA amplification. Use this chart to determine which product will work best for your needs.

	5′ → 3′ EXO ACTIVITY	AMPLIFICATION SPEED	ROOM TEMPERATURE SETUP	REVERSE TRANSCRIPTASE ACTVITY	INHIBITOR TOLERANCE	APPLICATIONS
***Bst* DNA Polymerase, Full Length**	★★	N/A	N/A	N/A	★	Nick translation reactions at elevated temperatures
***Bst* DNA Polymerase, Large Fragment**	N/A	★	N/A	★	★	General strand-displacement reactions, original polymerase for LAMP and other diagnostic amplifications
***Bst* 2.0 DNA Polymerase**	N/A	★★	N/A	★★	★★	Improved LAMP, SDA, and other amplification reactions
***Bst* 2.0 WarmStart DNA Polymerase**	N/A	★★	★★★	★★	★★	Consistent, room-temperature, and high-throughput amplification assays
***Bst* 3.0 DNA Polymerase**	N/A	★★★	★★	★★★	★★★	Fastest, most robust LAMP and RT-LAMP reactions. High reverse transcriptase activity up to 72°C

★★★ Optimal, recommended product for selected application
★★ Works well for selected application
★ Will perform selected application, but is not recommended
N/A Not applicable to this application

Choose from our selection of products

for your isothermal application.

PRODUCT	NEB #	SIZE
WarmStart LAMP KIT (DNA & RNA)	E1700S/L	100/500 reactions
WarmStart Colorimetric LAMP 2X Master Mix (DNA & RNA)	M1800S/L	100/500 reactions
Bst 3.0 DNA Polymerase	M0374S/L/M	1,600/8,000/8,000 units
Bst 2.0 WarmStart DNA Polymerase	M0538S/M/L	1,600/8,000 units
Bst 2.0 DNA Polymerase	M0537S/M/L	1,600/8,000 units
Bst DNA Polymerase, Large Fragment	M0275S/M/L	1,600/8,000 units
Bst DNA Polymease, Full Length	M0328S/L	500/2,500 units
WarmStart RTx Reverse Transcriptase	M0380S/L	50/250 reactions
Nt.BstNBI	R0607S/L	1,000/5,000 units
COMPANION PRODUCTS		
IsoAmp® II Universal tHDA Kit	H0110S	50 reactions
AMV Reverse Transcriptase	M0277S/T/L	200/500/1,000 units
Antarctic Thermolabile UDG	M0372S/L	100/500 units
Deoxynucleotide (dNTP) Solution Mix	N0447S/L	8/40 µmol of each
Deoxynucleotide (dNTP) Solution Set	N0446S	25 µmol of each

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