

## The Easy Way to Customize Your Protease Inhibitor Cocktail

◆ **Effective Concentrations**

◆ **Recipes for Stock and Working Solutions**

◆ **New! Protease Inhibitor Cocktail Sets**

◆ **Recommended Storage Conditions**

Cat. No.	Inhibitor	Target Protease Class/ Mechanism of Action	Effective Concentrations	Notes	Stock Solutions	Working Concentrations
110175	<b>Acetyl-Pepstatin</b> M.W. 643.8	<b>Aspartic/Reversible</b> Inhibits HIV-1 and HIV-2 proteases.	50 – 200 nM	Soluble in 50% acetic acid. $K_i$ for HIV-1 and HIV-2 proteases is 20 nM and 5 nM, respectively, at pH 4.7.	1 mg /1.553 ml of 50% acetic acid = 1 mM solution	Dilute 1:1000 to obtain 1 $\mu$ M concentration.
101500	<b>AEBSF</b> M.W. 239.5	<b>Serine/Irreversible</b> Inhibits trypsin, chymotrypsin, plasmin, thrombin, and kallikrein.	< 1 mM	Solutions in H <sub>2</sub> O are stable for 1 - 2 months at 4°C, pH 7. Will slowly hydrolyze at pH > 8.	50 mg/2.09 ml H <sub>2</sub> O = 100 mM solution	Use at 1 mM or less.
129875	<b>Amastatin</b> M.W. 474.6	<b>Metallo/Reversible</b> Inhibits alanyl-aminopeptidase.	1 - 10 $\mu$ M	Aqueous solutions are stable for 1 day. Stock solutions 1 mM in EtOH are stable for 1 month at -20°C.	1 mg/2.1 ml anhydrous EtOH or 5 mg/10.5 ml anhydrous EtOH = 1 mM	Dilute 1:100 for a 10 $\mu$ M working solution.
178220	<b>Antipain, Dihydrochloride</b> M.W. 677.6	<b>Serine and Cysteine/Reversible</b> Inhibits papain and trypsin. Plasmin is inhibited to a small extent. More specific than leupeptin for papain and trypsin.	1 - 100 $\mu$ M	Stable for several hours. Stock solutions of 10 mM in H <sub>2</sub> O or buffer are stable for 1 week at 4°C, 1 month at -20° C. Also soluble in MeOH and DMSO.	5 mg/8.27 ml H <sub>2</sub> O = 10 mM	Dilute 1:100 for a 100 $\mu$ M working solution.
169756	<b>Antithrombin III</b> M.W. 65,000	<b>Serine/Reversible</b> Inhibits thrombin, factor Xa, trypsin, and other trypsin-like serine proteases.	Use at equimolar concentration.	Aqueous stock solutions are stable at -20°C.	1 mg in 17.24 ml of 150 mM NaCl, 100 mM sodium citrate, 10 mM Tris-HCl, pH 8.3 = 1 mM	Use at concentration equimolar with protease.
178281	<b>p-APMSF Hydrochloride</b> M.W. 252.7	<b>Serine/Irreversible</b> Inhibits trypsin-like serine proteases. No effect on acetylcholinesterase.	10 - 100 $\mu$ M	Must be prepared fresh. Half-life of 6 min in pH 7.0 buffer systems. Stock solutions of 50 mM in H <sub>2</sub> O are stable when aliquoted and stored at -20°C.	5 mg/346 $\mu$ l in H <sub>2</sub> O = 50 mM	Dilute 1:500 for a 100 $\mu$ M solution.
616398 616399	<b>Aprotinins, Bovine Lung</b> M.W. 6512	<b>Serine/Reversible</b> Inhibits serine proteases, including plasmin, kallikrein, trypsin, and chymotrypsin. Does not inhibit thrombin or factor Xa.	0.6 - 2 $\mu$ g/ml	Very stable. Inactive at pH > 12.8. Very soluble in H <sub>2</sub> O (10 mg/ml). Aliquots are stable at -20°C.	10 mg/ml in PBS	Dilute 1:5000 for a 2 $\mu$ g/ml final concentration.
182525	<b>Arphamenine A</b> M.W. 387.4	<b>Metallo</b> A highly specific inhibitor of aminopeptidase B.	100 - 500 nM	Soluble in water. Stock solutions are stable for up to 1 month at -20°C.	1 mg/2.58 ml of H <sub>2</sub> O = 1 mM solution	Dilute 1:1000 to obtain 1 $\mu$ M concentration.
182530	<b>Arphamenine B</b> M.W. 387.4	<b>Metallo</b> A highly specific inhibitor of aminopeptidase B.	50 - 100 nM	Soluble in water. Stock solutions are stable for up to 1 month at -20°C.	1 mg/2.48 ml of H <sub>2</sub> O = 1 mM solution	Dilute 1:1000 to obtain 1 $\mu$ M concentration.
200484	<b>Bestatin</b> M.W. 308.4	<b>Metallo/Reversible</b> Inhibits alanyl-aminopeptidase.	1 - 10 $\mu$ M	Stable for 1 day. Stock solutions of 1 mM in MeOH are stable for at least 1 month at -20°C.	2 mg/6.48 ml MeOH = 1 mM	Dilute 1:100 for 10 $\mu$ M working solution.
03-34-0051	<b>Calpeptin</b> M.W. 362.5	<b>Cysteine</b> Cell-permeable inhibitor of calpain I and II and papain.	0.3 - 1.0 $\mu$ M	A cell-permeable calpain inhibitor. Soluble in DMSO and DMF. Dilute in aqueous medium just before use.	5 mg/13.8 ml DMSO = 1 mM solution	Dilute 1: 1000 to obtain 1 $\mu$ M concentration.

Cat. No.	Inhibitor	Target Protease Class/ Mechanism of Action	Effective Concentrations	Notes	Stock Solutions	Working Concentrations
219415	<b>Cathepsin Inhibitor I</b> M.W. 475.5	<b>Cysteine/Irreversible</b> Inhibits cathepsin B, cathepsin L, cathepsin S, and papain.	10 - 100 $\mu$ M	Protect from light. Half-life in a buffered solution (pH 5 - 9) is 25 hours at 30°C and about 2 weeks at -20°C.	1 mg/210 $\mu$ l DMSO or EtOH = 10 mM solution	Dilute 1:50 to obtain 200 $\mu$ M concentration.
219417	<b>Cathepsin Inhibitor II</b> M.W. 489.5	<b>Cysteine/Irreversible</b> Inhibits cathepsin B, cathepsin L, cathepsin S, and papain.	10 - 100 $\mu$ M	Protect from light. Half-life in a buffered solution (pH 5 - 9) is 27 hours at 30°C and about 2 weeks at 0°C.	1 mg/204 $\mu$ l DMSO or EtOH = 10 mM solution	Dilute 1:50 to obtain 200 $\mu$ M concentration.
219419	<b>Cathepsin Inhibitor III</b> M.W. 505.5	<b>Cysteine/Irreversible</b> Inhibits cathepsin B, cathepsin L, cathepsin S, and papain.	10 - 100 $\mu$ M	Protect from light. Half-life in a buffered solution (pH 5 - 9) is 37 hours at 30°C and about 2 weeks at 0°C.	1 mg/198 $\mu$ l DMSO or EtOH = 10 mM solution	Dilute 1:50 to obtain 200 $\mu$ M concentration.
219420	<b>Cathepsin/Subtilisin Inhibitor</b> M.W. 518.0	<b>Cysteine and Serine/Irreversible</b> Inhibits cathepsin L, subtilisin, and thermitase.	10 - 100 $\mu$ M	Protect from light. Half-life in a buffered solution (pH 5 - 9) is 2.5 hours at 30°C and about 40 hours at 0°C.	1 mg/193 $\mu$ l DMSO or EtOH = 10 mM solution	Dilute 1:100 to obtain 100 $\mu$ M concentration.
230790	<b>Chymostatin</b> M.W. 604.9	<b>Cysteine and Serine/Reversible</b> Inhibits chymotrypsin-like serine proteases including $\alpha$ -, $\beta$ -, $\gamma$ -, and $\delta$ -chymotrypsin.	10 - 100 $\mu$ M	Stable for several hours. Soluble in DMSO and glacial acetic acid. Solutions are stable for months at -20°C.	5 mg/825 $\mu$ l in DMSO = 10 mM solution	Dilute 1:100 for a 100 $\mu$ M working solution.
240891	<b>Cystatin, Egg White</b> M.W. 12,700	<b>Cysteine/Reversible</b> Inhibits cysteine proteases, including dipeptidyl peptidase I and III, papain, ficin, and cathepsin B.	Use at equimolar concentrations.	Very stable. Stable to heat. Freeze in the presence of 20% glycerol or buffered to pH 7.5.	At least 1 mg/ml in 20 mM Tris, pH 7.5	Use at equimolar concentration.
287815	<b>3,4-Dichloroisocoumarin</b> M.W. 215.0	<b>Serine/Irreversible</b> Inhibits a wide range of serine proteases. Not active towards $\beta$ -lactamases.	5 - 100 $\mu$ M	Half-life of 20 minutes at pH 7.5. Stock solutions of 10 mM in DMF or DMSO are stable for months at -20°C.	10 mg/4.65 ml in DMSO = 10 mM solution	Dilute 1:100 for 100 $\mu$ M working solution.
30967	<b>DFP</b> M.W. 184.2	<b>Serine/Irreversible</b> Inhibits serine proteases. Inactivates acetylcholinesterase.	100 $\mu$ M	Very toxic: special handling required. Half-life of 1 hour at pH 7.5. Stock solutions of 0.2 - 0.5 M in dry propanol stable for several months at -70°C. <b>Not for international sale.</b>	1 g/10.86 ml in dry propanol = 0.5 M	Dilute 1:5000 for 100 $\mu$ M working solution.
416200	<b>Dipeptidylpeptidase IV Inhibitor I</b> M.W. 455.5	<b>Serine/Proline Specific</b> Inhibits dipeptidylpeptidase IV.	10 - 100 $\mu$ M	DMSO stock solutions are stable for up to 6 months at 20°C. Use only high quality, moisture free, DMSO. Aqueous solutions are stable for 1 day.	1 mg/219.5 $\mu$ l DMSO = 10 mM solution	Dilute 1:100 to obtain a 100 $\mu$ M working solution.
317638	<b>Dipeptidylpeptidase IV Inhibitor II</b> M.W. 355.8	<b>Serine/Proline Specific/Irreversible</b> Acylation inhibitor of dipeptidylpeptidase II and IV.	1 - 10 $\mu$ M	Protect from light. Half-life in a buffered solution (pH 7.6) is 24 hours at 30°C.	1 mg/2.81 ml DMSO or EtOH = 1mM solution	Dilute 1:100 to obtain 10 $\mu$ M concentration.
03-34-0012	<b>Diprotin A</b> M.W. 341.5	<b>Metallo/Reversible</b> Inhibits dipeptidylpeptidase IV.	10 - 50 $\mu$ M	Stable for 1 day at working concentrations. Stock solutions can be prepared 1 mM in water, MeOH, or EtOH. Stability has not been determined.	2 mg /5.86 ml = 1 mM solution	Dilute 1:20 for a 50 $\mu$ M working solution.
330005	<b>EST</b> M.W. 342.4	<b>Cysteine/Irreversible</b> Cell-permeable inhibitor of lysosomal cysteine proteases.	20 - 50 $\mu$ g/ml	A membrane-permeable calpain inhibitor. Stock solutions are stable for up to 6 months at -20°C.	1 mg/ml in EtOH	Use 20 to 50 $\mu$ l per ml of medium.
324890	<b>E-64 Protease Inhibitor</b> M.W. 357.4	<b>Cysteine/Irreversible</b> Inhibits cysteine proteases. Does not affect cysteine residues in other enzymes or react with low molecular weight thiols such as $\beta$ -mercaptoethanol. Very specific, active site titrant.	1 - 10 $\mu$ M	Stable for days at neutral pH. Stock solutions of 1 mM in aqueous solutions are stable for months at -20°C.	5 mg/13.99 ml = 1 mM solution	Dilute 1:100 for a 10 $\mu$ M working solution.
324475	<b>Ebelactone A, Streptomyces</b> M.W. 380.3	Inhibits esterase, lipase, and N-formylmethionine aminopeptidase.	1 - 2 $\mu$ g/ml	Soluble in MeOH (200 mg/ml), EtOH, and $\text{CHCl}_3$ . Solutions are stable for 1 week at 4°C and 1 month at -20°C.	1 mg/2.95 ml = 1 mM solution	Use 2.6 or 5.2 $\mu$ l/ml for 1 or 2 $\mu$ g, respectively.
324478	<b>Ebelactone B, Streptomyces</b> M.W. 352.5	<b>Serine</b> A specific inhibitor of carboxypeptidase Y-like exopeptidase.	0.1 - 2 mg/ml	Soluble in MeOH (200 mg/ml), EtOH, and $\text{CHCl}_3$ . Solutions are stable for 1 week at 4°C and 1 month at -20°C.	1 mg/2.84 ml = 1 mM solution	Use 2.84 or 5.08 $\mu$ l/ml for 1 or 2 $\mu$ g, respectively.
330200	<b>Ecotin</b> M.W. 32,200	<b>Serine</b> A broad range serine protease inhibitor. Inhibits chymotrypsin, elastase, Factors Xa and XIIa, and trypsin.	0.5 to 1.5 nM	Avoid freeze/thaw cycles. Store the stock solution at -70°C.	Provided as a solution in 1 mM HCl	Check for lot specific volume information and dilute accordingly.
34103	<b>EDTA, Tetrasodium Salt</b> M.W. 380.2	<b>Metallo/Reversible Chelator</b> May interfere with other metal-dependent biological processes.	1 - 10 mM	Stable in aqueous solution. Stock solutions of 0.5 M in $\text{H}_2\text{O}$ , pH 8.5, are stable for several months at 4°C.	1.9 g/10 ml = 0.5 M solution	Dilute 1:50 for a 10 mM working solution.
324691	<b>Elastinal</b> M.W. 512.6	<b>Serine/Reversible</b> Inhibits elastase and elastase-like serine proteases.	10 - 100 $\mu$ M	Stable for several hours. Stock solutions of 10 mM in $\text{H}_2\text{O}$ are stable for 1 week at 4°C and for several months at -20°C.	5 mg/975 $\mu$ l = 10 $\mu$ M solution	Dilute 1:100 for a 100 $\mu$ M working solution.
432077	<b>Leuhistin</b> M.W. 241.3	<b>Metallo/Reversible</b> Competitive inhibitor of aminopeptidase M.	1 - 2 $\mu$ g/ml	Soluble in water, EtOH, and DMSO. Store stock solutions at -20°C.	1 mg/ml of $\text{H}_2\text{O}$	Use 1 to 2 $\mu$ l/ml.
108975	<b>Leupeptin, Hemisulfate, Dihydrate</b> M.W. 493.6	<b>Serine and Cysteine/Reversible</b> Inhibits trypsin-like proteases and some cysteine proteases including endoproteinase Lys-C, kallikrein, papain, thrombin, cathepsin B, and trypsin.	10 - 100 $\mu$ M	Stable for several hours. Stock solutions of 10 mM in $\text{H}_2\text{O}$ are stable for 1 week at 4°C and 1 month at -20°C.	5 mg/1.054 ml = 10 mM solution	Dilute 1:100 for a 100 $\mu$ M working solution.
441251	<b><math>\alpha_2</math>-Macroglobulin, Human Plasma</b> M.W. 725,000	<b>Broad Range/Irreversible</b> Inhibits bromelain, chymotrypsin, elastase, endoproteinase Arg-C, endoproteinase Glu-C, ficin, papain, plasmin, subtilisin, thermolysin, thrombin, and trypsin. Forms "trap" around most proteases.	Use at equimolar concentrations.	Aqueous stock solutions are stable for 6 months at -20°C at pH 6 - 7.5. Sensitive to acidic pH; denatured below pH 4.0. Ammonia, methylamine, and hydroxylamine (above pH 7.0) cause irreversible conversion to the closed form. Do not use in the presence of DTT.	1 mg/13.8 $\mu$ l = 100 $\mu$ M solution	Use at equimolar concentrations.
479919	<b>NCO-700</b> M.W. 1141.3	<b>Cysteine</b> Inhibits calpain, cathepsin B, cathepsin L, and papain.	0.5 - 100 mM	Prepare a solution on the day of use. In aqueous solution, this material is stable for up to 6 hours at room temperature. Stable for longer periods in EtOH.	1 mg/87.6 ml PBS, $\text{H}_2\text{O}$ or EtOH = 10 mM solution	Dilute 1:100 to obtain a 100 $\mu$ M working solution.
516482	<b>Pepstatin</b> M.W. 685.9	<b>Aspartic/Reversible</b> Inhibits cathepsin D, cathepsin G, pepsin, renin, and many microbial aspartic proteases. Weak binding to proteases.	1 $\mu$ M	Soluble in MeOH to 1 mg/ml. Soluble to 1 mg/ml in EtOH if allowed to sit overnight and to 333 mg/ml in 6 N acetic acid. Stable for 1 week at 4°C.	5 mg/7.29 ml = 1 mM solution	Dilute 1:1000 for 1 $\mu$ M working solution.
525276	<b>Phosphoramidon, Ammonium Salt</b> M.W. 579.6	<b>Metallo/Reversible</b> Inhibitor of some metalloendopeptidases. A highly specific inhibitor of thermolysin.	25 mg/ml (8.5 mM)	Stock solutions of 1 mg/ml in $\text{H}_2\text{O}$ are stable for at least 1 month at -20°C.	1 mg/1.015 ml = 1.7 mM solution	Dilute 1:200 for a 8.5 $\mu$ M working solution.
52332	<b>PMSF</b> M.W. 174.2	<b>Serine/Irreversible</b> Inhibits carboxypeptidase Y, chymotrypsin, factor Xa, papain, plasmin, proteinase K, subtilisin, thrombin, and trypsin.	0.1 - 1 mM	Toxic: Must be prepared fresh and added at several steps during sample preparation. Half-life of 1 hour at pH 7.5. Stock solutions of 200 mM in anhydrous solvents (MeOH, EtOH) are stable for at least 9 months at 4°C.	100 mg/2.87 ml = 200 mM solution	Dilute 1:200 for a 1 $\mu$ M working solution.
572915	<b>Subtilisin Inhibitor I</b> M.W. 379.4	<b>Serine</b> Inhibits subtilisin and thermitase.	10 - 100 $\mu$ M	Protect from light. Half-life in a buffered solution (pH 5 - 9) is 4 hours at 30°C; 60 hours at 0°C.	1 mg/264 $\mu$ l DMSO or EtOH = 10 mM solution	Dilute 1:100 to obtain 100 $\mu$ M concentration.
572917	<b>Subtilisin Inhibitor II</b> M.W. 475.5	<b>Serine</b> Inhibits subtilisin and thermitase.	10 - 100 $\mu$ M	Protect from light. Half-life in a buffered solution (pH 5 - 9) is 4 hours at 30°C; 60 hours at 0°C.	1 mg/210.3 $\mu$ l DMSO or EtOH = 10 mM solution	Dilute 1:100 to obtain 100 $\mu$ M concentration.
572920	<b>Subtilisin Inhibitor III</b> M.W. 505.5	<b>Serine</b> Inhibits subtilisin and thermitase.	10 - 100 $\mu$ M	Protect from light. Half-life in a buffered solution (pH 5 - 9) is 8 hours at 30°C; 120 hours at 0°C.	1 mg/198 $\mu$ l DMSO or EtOH = 10 mM solution	Dilute 1:100 to obtain 100 $\mu$ M concentration.
572922	<b>Subtilisin Inhibitor IV</b> M.W. 516.0	<b>Serine</b> Inhibits subtilisin.	10 - 100 $\mu$ M	Protect from light. Half-life in a buffered solution (pH 5 - 9) is 6.5 hours at 30°C; 100 hours at 0°C.	1 mg/194 $\mu$ l DMSO or EtOH = 10 mM solution	Dilute 1:100 to obtain 100 $\mu$ M concentration.
572925	<b>Subtilisin Inhibitor V</b> M.W. 552.6	<b>Serine/Cysteine/Irreversible</b> Inhibits subtilisin and elastase.	10 - 100 $\mu$ M	Protect from light. Half-life in a buffered solution (pH 5 - 9) is 4 hours at 30°C; 60 hours at 0°C.	1 mg/181 $\mu$ l DMSO or EtOH = 10 mM solution	Dilute 1:100 to obtain 100 $\mu$ M concentration.

Ask us about our wide selection of Inhibitors of Calpains, Caspases, and Proteasomes.

Cat. No.	Inhibitor	Target Protease Class/ Mechanism of Action	Effective Concentrations	Notes	Stock Solutions	Working Concentrations
616382	<b>TLCK</b> M.W. 369.3	<b>Serine/Irreversible</b> Inhibits trypsin-like serine proteases including bromelain, endoproteinase Arg-C, endoproteinase Lys-C, ficin, papain, plasmin, thrombin, and trypsin.	10 - 100 $\mu$ M	Very unstable above pH 7.5. Stock solutions of 10 mM in aqueous solutions (1 mM HCl, pH 3.0) or MeOH should be prepared fresh as needed.	5 mg/1.354 ml = 10 mM solution	Dilute 1:100 for a 100 $\mu$ M working solution.
616387	<b>TPCK</b> M.W. 351.5	<b>Serine/Irreversible</b> Inhibits chymotrypsin-like serine proteases including bromelain, chymotrypsin, ficin and papain.	10 - 100 $\mu$ M	Stable for several hours. Stock solutions of 10 mM in MeOH are stable for several months at 4°C.	5 mg/1.42 ml = 10 mM solution	Dilute 1:100 for a 100 $\mu$ M working solution.
65035 650357	<b>Trypsin Inhibitor, Soybean</b> M.W. 20,000	<b>Serine/Reversible</b> Inhibits factor Xa and trypsin. Cat. No. 650357 is a high activity form.	Use at equimolar concentrations.	Dissociated at low pH. Stock solutions in dilute buffers are stable at -20°C.	20 mg/ml = 1 mM solution	Use at equimolar concentrations.

*We also carry a Protease Inhibitor Set and 3 Specially Formulated Cocktails for easier use!*

### Protease Inhibitor Set Cat. No. 539128

Contains 50 mg of AEBSF, HCl (Cat. No. 101500), 1 mg of E-64 (Cat. No. 324890), 1 mg of EST (E-64d; Cat. No. 330005), 5 mg of Leupeptin, Hemisulfate (Cat. No. 108975), 5 mg of Pepstatin A (Cat. No. 516482), 50 mg of TLCK (Cat. No. 616382) and 250 mg of TPCK (Cat. No. 616387).

### Protease Inhibitor Cocktail Set II Cat. No. 539132

A cocktail of 5 protease inhibitors with broad specificity to the inhibition of aspartic, cysteine, serine, and metalloproteases as well as aminopeptidases. Recommended for use with bacterial cell extracts. *Reconstitute each vial with 1 ml DMSO (provided) and 4 ml of H<sub>2</sub>O to obtain a 5 ml stock solution. Each vial contains 20 mM AEBSF (Cat. No. 101500), 1.7 mM Bestatin (Cat. No. 200484), 200  $\mu$ M E-64 (Cat. No. 324890), 85 mM EDTA, and 2 mM Pepstatin A (Cat. No. 516482).* Supplied as 1 set (1 vial of cocktail mixture plus one vial of DMSO) or 5 sets (5 vials of cocktail mixture plus 5 vials of DMSO).

### Protease Inhibitor Cocktail Set I Cat. No. 539131

A cocktail of five protease inhibitors that will inhibit a broad range of proteases. *Reconstitute each vial with 1 ml H<sub>2</sub>O to obtain a 100X stock solution. 1X stock solution contains 500  $\mu$ M AEBSF, HCl (Cat. No. 101500), 1  $\mu$ g/ml Aprotinin (Cat. No. 616398), 1  $\mu$ M E-64 (Cat. No. 324890), 500  $\mu$ M EDTA and 1 mM Leupeptin (Cat. No. 108975).* Supplied as one vial or a set of 10 vials.

### Protease Inhibitor Cocktail Set III Cat. No. 539134

A cocktail of six protease inhibitors with broad specificity for the inhibition of aspartic, cysteine, and serine proteases as well as aminopeptidases. Provided as a DMSO solution. Recommended for use with mammalian cell and tissue extracts. *Each vial contains 100 mM AEBSF (Cat. No. 101500), 80  $\mu$ M Aprotinin (Cat. No. 616398), 5 mM Bestatin (Cat. No. 200484), 1.5 mM E-64 (Cat. No. 324890), 2 mM Leupeptin (Cat. No. 108975), and 1 mM Pepstatin A (Cat. No. 516482).* Supplied as 1 vial or as a set of 5 vials.

Please call our Technical Service Department or your local sales office for more information on these products.

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