User Guide

SMC® Human IL-17A High Sensitivity Immunoassay Kit

Microparticle Assay

Human IL-17A High Sensitivity Immunoassay Kit for the Quantitative Determination of IL-17A in Human Plasma and Serum

03-0159-00

Introduction

The SMC® Human IL-17A High Sensitivity Immunoassay uses a quantitative fluorescent sandwich immunoassay technique to measure IL-17A in human plasma and serum samples. A capture antibody specific for human IL-17A has been pre-coated onto paramagnetic microparticles (beads). The user pipettes beads, standards, and samples into uncoated microplate wells. During incubation, the IL-17A present in the sample binds to the capture antibody on the coated beads. Unbound molecules are washed away during the subsequent wash steps. Fluor-labeled detection antibody is added to each well and incubated. This detection antibody recognizes and binds to IL-17A that has been captured onto the beads, thus completing the immunosandwich. Elution buffer is then added and incubated. The elution buffer dissociates the bound protein sandwich from the beads surface releasing the labeled antibodies. The eluted antibodies are transferred to a SMC® 384-well Read Plate. The plate is loaded into the SMCxPRO® Immunoassay System where the labeled molecules are detected and counted. The number of fluor-labeled detection antibodies counted is directly proportional to the amount of IL-17A present in the sample when captured. The amount of IL-17A in unknown samples is interpolated from a standard curve.

Supplies

The SMC® Human IL-17A Immunoassay Kit includes all reagents listed below; these components are lot matched and not intended to be used separately. Additional reagents and supplies are required to run this immunoassay, as listed in the next section, Additional Supplies Required (Not provided).

This kit and all reagents supplied are for research use only.

Reagents Included with the Kit

All items are shipped with a cold pack unless otherwise stated.

Description	Storage Conditions	Packaging Details	Component Part No.
Assay Buffer	2-8 °C	2 x 20 mL	02-0357-01
IL-17A Beads	2-8 °C	1 x 550 µL	02-2159-00
Standard Diluent	2-8 °C	2 x 20 mL	02-0225-02
IL-17A Detection Antibody	2-8 °C	1 x 270 µL	02-1159-00
IL-17A Standard	2-8 °C	1 lyophilized vial	02-8159-00
10X Wash Buffer	2-8 °C	1 x 50 mL	02-0001-03
Buffer D	2-8 °C	1 x 6 mL	02-0446-00
Elution Buffer B	2-8 °C	1 x 5 mL	02-0211-02

Storage Instructions

The SMC® Human IL-17A High Sensitivity Immunoassay Kit should be stored at 2-8 °C.

Discard standards after one use.

Supplied 10X Wash Buffer does not contain preservative. After dilution, the 1X Wash Buffer may be filter sterilized with Stericup® Filter for storage of up to 1 month at 2-8 °C. If not filter sterilized, all remaining 1X Wash Buffer should be discarded upon experiment completion.

Proper kit performance can only be guaranteed if the materials are stored properly.

Additional Supplies Required (Not provided)

Catalogue numbers are provided to purchase products at SigmaAldrich.com or through sales quote, unless otherwise noted.

Equipment

- SMCxPRO® Ultrasensitive Immunoassay System for sample acquisition (95-0100-00)
- Orbital microplate shaker for assay plate incubation (for example, Boekel Scientific Jitterbug™ Shaker)
- Bio-Tek® 405 TSUVS Microplate Washer for assay plate washing (95-0004-05)
- Sphere Mag Plate for performing microparticle capture (90-0003-02)
- Rotisserie tube rotator for microparticle suspension
- Benchtop centrifuge with bucket rotors capable of reaching 1,100 x *g* for sample/plate centrifugation
- Microcentrifuge capable of reaching 13,000 x *g* for reagent/sample centrifugation
- Single channel manual pipettes to accurately dispense 10-20 µL and 20-250 µL
- 12-channel manual pipettes to accurately dispense 10-20 µL and 20-250 µL
- Plate roller for complete plate sealing (Fisher Scientific, NC9185793)

Supplies

- Micro-centrifuge tubes for sample preparation and storage
- 1 L Container with cap for Wash Buffer dilution
- Stericup® Quick Release Vacuum Filtration System, 0.22 µm, 1 L; for filter sterilizing 1X Wash Buffer (S2GPU11RE)
- MultiScreen®$_{HTS}$ 96-well Plate, hydrophilic PVDF membrane (MSBVN1210)
- 15 mL conical tube with cap for capture bead and detection antibody dilution
- 96-well V-bottom plate for assay setup (AXYP96450VCS)
- Axygen™ Microplate Sealing Film and Tapes (Fisher Scientific, 14-222-344)
- Universal plate cover to minimize plate well contamination (Fisher Scientific, 253623)
- 12-Channel reagent reservoir (sterile) for standard serial dilution (Argos/Cole Parmer, 04395-33)
- VistaLab® 25 mL Reservoirs for addition of reagents (Fisher Scientific, 21-381-27C)
- Millex® Syringe Filter, 0.2 µm for detection antibody filtration (SLGPR33RS)
- Luer-Lok® Syringe, 5 mL; for Detection Antibody Filtration (Fisher Scientific, 14-829-45)
- Nunc™ Aluminum adhesive plate seals (Fisher Scientific, 276014)
- SMC® 384-well plate with adhesive seal (02-1008-00)
- SMC® 384-well plate, bulk case of 32 (ABB2-00160A)

Reagents

- 10X Wash Buffer for automated assay plate washing, 1 L (02-0111-00)
- De-ionized or distilled water for dilution of 10X Wash Buffer

Assay Best Practices

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. In addition, proper training as well as instrument maintenance is critical for obtaining optimal results in performing SMC® assays. The following notes should be reviewed and understood before the assay is set up.

- Wipe down bench and pipettes with 70% isopropanol before use.
- It is important to allow all reagents to warm to room temperature (RT), 20-25 °C.
- Use sterile filter pipette tips and reagent trays to avoid contamination.
- Pre-wet tips (aspirate and dispense within well) twice before each transfer.
- The standards prepared by serial dilution must be used within 10 minutes of preparation.

  Note: It is recommended that the standards are prepared as the last step prior to plate setup.
- All washing must be performed with the Wash Buffer provided.
- An orbital microplate shaker for assay plate incubation (Jitterbug™ Shaker, settings #3-5) provide maximal orbital mixing without splashing liquid or causing cross-contamination.

  Jitterbug™ Shaker setting #3 ~ 750 rpm

  Jitterbug™ Shaker setting #4 ~ 875 rpm

  Jitterbug™ Shaker setting #5 ~1000 rpm

  Note: If using different orbital shaker, refer to recommended rpm ranges provided for each incubation step, and adjust speeds as necessary to ensure maximal orbital mixing without splashing liquid or causing cross-contamination.
- As the SMC® assay is extremely sensitive to dust particles, do not perform the assay or plate washing under direct airflow.
- Plate must also be protected from light after adding detection.
- After the assay is complete, seal the plate before reading immediately or storing temporarily at 2-8 °C. The SMCxPRO® Immunoassay System requires the use of aluminum adhesive plate seal.
- It is not recommended to store eluted products from SMC® assays overnight at 4 °C or frozen at –80 °C for later reading as performance cannot be guaranteed.
- If SMC® Read Plate has been stored at 4 °C, plate should be left at RT for 30 minutes to 1 hour on the benchtop before reading to avoid a rapid increase in temperature within SMC® Read Plate wells. Bring to RT then centrifuge the plate at 1,100 x *g* for 1 minute prior to reading.
- For optimal SMCxPRO® Immunoassay System performance, perform ASSIST testing daily (ideally at beginning of the day before assay is prepared).

Precautions

Use caution when handling biological samples. Wear protective clothing and gloves. Components of this reagent kit contain Sodium azide as a preservative. Sodium azide is a toxic and dangerous compound when combined with acids or metals. Solutions containing Sodium azide should be disposed of properly.

Ingredient	Catalogue Number	Full Label	
IL-17A Standard	02-8159-00	 	**Warning.** Harmful if swallowed. May cause damage to organs like, respiratory tract through prolonged or repeated exposure if inhaled. Do not breathe dust/ fume/ gas/ mist/ vapours/ spray. Wash skin thoroughly after handling. Do not eat, drink or smoke when using this product. IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. Get medical advice/ attention if you feel unwell. Rinse mouth. Dispose of contents/ container to an approved waste disposal plant.
IL-17A Beads	02-2159-00	No symbol required.	Harmful to aquatic life with long lasting effects. Avoid release to the environment.
Assay Buffer	02-0357-01	No symbol required.	Harmful to aquatic life with long lasting effects. Avoid release to the environment.
Standard Diluent	02-0225-02		**Warning**. May cause damage to organs like, respiratory tract through prolonged or repeated exposure if inhaled. Do not breathe dust/ fume/ gas/ mist/ vapours/ spray. Get medical advice/ attention if you feel unwell. Dispose of contents/ container to an approved waste disposal plant.
10X Wash Buffer	02-0001-03		**Warning.** Causes serious eye irritation. Harmful to aquatic life with long lasting effects. Avoid release to the environment. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Assay Preparation

Reagent Preparation

1. Warm all reagents to RT prior to use.
2. Store the Detection Antibody away from light until ready to use.
3. Prepare 1X Wash Buffer (from 10X Wash Buffer) as follows:

   Pour 50 mL of 10X Wash Buffer into a container capable of holding at least 500 mL. Add 450 mL of deionized water.

   Mix thoroughly by gentle inversion or with a clean, sterile stir bar.

   Note: 1X Wash Buffer may be filter sterilized.
4. Mix IL-17A Antibody Coated Beads on a rotisserie spin rotator, or manually by repeat inversion, for ≥ 20 minutes until all beads are resuspended.

Sample Preparation

1. Prepare samples by one of the following methods:

   If using a microcentrifuge: Centrifuge samples at > 13,000 x *g* for 10 minutes immediately prior to use. Carefully pipette the supernatant into a clean microcentrifuge tube, avoiding particulates and slowly aspirating below the lipid layer.

   If using a filter plate with prefilter: Stack the filter plate on top of a 96-well receptacle plate. Place 250 µL of sample into a filter plate well and spin for ≥ 10 minutes at 1,100 x *g*.
2. Dilute the clarified samples 1:2 using the Standard Diluent. (For triplicates, transfer 200 µL of clarified sample to the sample preparation plate and add 200 µL Standard Diluent).

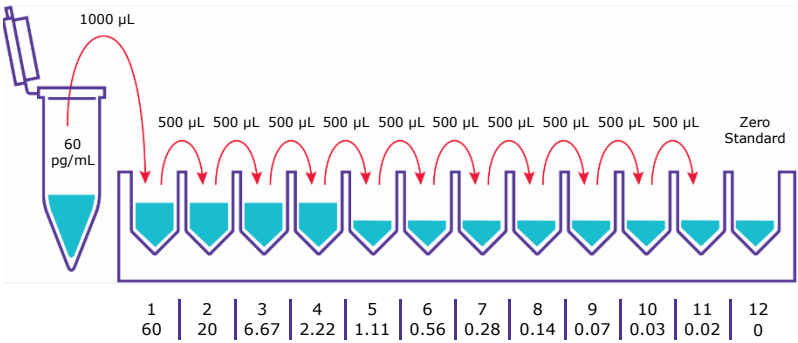
Initial Standard Stock Preparation

1. Reconstitute lyophilized standard in 250 µL of deionized water. Invert the vial several times to mix. Gently pulse vortex the vial for 10 seconds. Allow the vial to sit for 5-10 minutes.
2. Refer to the standard value assignment on the Certificate of Analysis for the starting concentration of the IL-17A Standard in the vial.
3. Perform the necessary dilutions in Standard Diluent to achieve the final working concentration of 60 pg/mL in a 1 mL final volume.

Standard Curve

Prepare the standard curve in a 12-channel reagent reservoir. Perform 1:3 serial dilutions of the 60 pg/mL Standard 1 for Standards 2 through 4 and 1:2 dilution of Standard 4 for Standards 5 through 11 to achieve a curve from 60 pg/mL to 0.02 pg/mL. Standard 12 is the Blank (Standard Diluent only).

Run the standards in triplicate.



1. Add 1000 µL Standard Diluent to wells 2 through 4 and 500 µL of Standard Diluent to wells 5 through 12 of a 12-channel reservoir dilution plate.
2. Transfer 1000 µL 60 pg/mL working stock (Standard 1) into well 1.
3. Transfer 500 µL from well 1 into well 2, mixing thoroughly. Continue serial dilutions from well 2 stopping at well 11, mixing thoroughly each time. Use a fresh tip with each transfer.

Assay Procedure

Target Capture

1. Pipette 100 µL per well of Standards or 1:2 Samples to assay plate.
2. Following mixing of the Coated Beads, immediately before adding to the assay plate, add the entire vial of Coated Beads to 11.0 mL of supplied Assay Buffer. Rinse bead vial with 0.55 mL of Assay Buffer and ensure that all beads have been transferred. Mix by gentle inversion. There should be a total volume of 12.1 mL of diluted Coated Beads.
3. Pipette 100 µL per well of the Coated Beads into assay plate.
4. Seal assay plate with clear adhesive plate seal, apply pressure to seal to prevent leaking and cross-contamination.
5. Incubate for 2 hours at 25 °C on microplate incubator/shaker (Jitterbug™ Shaker setting #4).
6. Approximately 10 minutes prior to the end of target capture incubation, prepare the Detection Antibody using one of the following methods:
   - Centrifuge 20X Detection Antibody at 14,000 x *g* for 5 minutes. Prepare 1X Detection Antibody by adding 250 µL of the centrifuged supernatant into 4,750 µL of Assay Buffer.
   - Prepare 1X Detection Antibody by adding 250 µL of 20X Detection Antibody into 4,750 µL of Assay Buffer and filter the diluted Detection Antibody using the syringe with a 0.2 µm filter into a clean tube.
7. When incubation is complete, centrifuge at 1,100 x *g* for 1 minute and carefully remove clear adhesive plate seal to avoid splashing.

Post-Capture Wash

Wash plate once with a plate washer (Bio-Tek® 405 TSUVS; Post Capture Wash (POSTCAP)). If using automation, please contact your technical service representative for the appropriate automation procedure.

Detection

1. After removal from plate washer, dispense 20 µL per well of Detection Antibody without disturbing the bead pellet (It is recommended to change tips).
2. Seal assay plate with clear adhesive plate seal.
3. Incubate for 1 hour at 25 °C on microplate incubator/shaker (Jitterbug™ Shaker setting #5). Ensure plate is protected from light during this incubation.
4. After incubation, carefully remove clear adhesive plate seal to avoid splashing.

Post-Detection Wash

Wash the assay plate 4 times with wash buffer using the 4 cycle Pre-Transfer (4CYCPRE) program on the Bio-Tek® 405 TSUVS washer. If using automation, please contact your technical service representative for the appropriate automation procedure.

Post-Detection Shake

1. After 4 cycle Pre-Transfer wash, visually verify that each well contains ~200 µL of Wash Buffer.
2. Seal assay plate with clear adhesive plate seal and apply pressure to the seal to prevent leaking and cross-contamination.
3. Place plate on microplate/incubator shaker for 2.0 minutes (Jitterbug™ Shaker setting #3).
4. Remove the plate from the microplate/incubator shaker, carefully remove clear adhesive plate seal to avoid splashing and place it on the plate washer to perform final aspiration.

Final Aspiration

Perform Final Aspiration using Bio-Tek® 405 TSUVS; Final Aspirate (FINASP). If using automation, please contact your technical service representative for the appropriate automation procedure.

Elution

1. Dispense 10 µL Elution Buffer B per well using reverse pipetting without disturbing the bead pellet (It is recommended to change tips).
2. Seal assay plate with a clear adhesive plate seal.
3. Incubate plate for 10 minutes at 25 °C on microplate incubator/shaker (Jitterbug™ Shaker setting #5).

Assay Reading

To read on the SMCxPRO® Immunoassay System

1. Add 10 µL per well of Buffer D using reverse pipetting to a fresh 96-well assay plate, using a 12-channel manual pipette (1-20 µL).
2. Place assay plate with Elution Buffer B onto sphere mag plate and allow beads to form a tight pellet for 2 minutes.
3. While keeping the assay plate containing eluate on sphere mag plate, gently remove clear adhesive seal and transfer 10 µL of eluate to the assay plate containing Buffer D by aspirating directly from the V-bottom of the plate, avoiding the pelleted beads, and changing tips with each dispensed row.
4. Seal this plate with a clear adhesive plate seal.
5. Place the plate (containing eluted, neutralized antibody solution) into microplate incubator/shaker and shake for 2 minutes at 25 °C (Jitterbug™ Shaker setting #5), centrifuge plate for 1 minute at RT, approximately 1,100 x *g*.
6. Gently remove clear adhesive plate seal and transfer 20 µL of neutralized eluate solution per well to corresponding wells of the SMC® Read Plate, placed over the included plate holder.
7. Seal SMC® Read Plate with clear adhesive plate seal. Centrifuge plate for 1 minute at RT, approximately 1,100 x *g*. Remove plate sealer, inspect SMC® Read Plate wells and remove bubbles if they are present.
8. Firmly seal SMC® Read Plate with aluminum adhesive plate seal using the recommended plate roller.
9. Remove the plate holder from the sealed SMC® Read Plate and load it onto the SMCxPRO® Immunoassay System. Start read.

   Note: There is a warmup period of up to 30 minutes to wait for the SMC® Read Plate to be close to the internal instrument temperature. Once achieved the read will start automatically.

SMC® Assay Overview

1. Prepare all reagents, standard curve, and samples as instructed.
2. Add 100 µL of Standard/1:2 Diluted Samples and 100 µL of Coated Beads to assay plate.
3. Seal and incubate for 2 hours at 25 °C on appropriate microplate incubator/shaker.

2 hours 25 °C

4. After capture incubation, centrifuge assay plate at 1,100 x *g* for 1 minute.
5. Perform Post-Capture Wash.
6. Remove from washer magnet and add 20 µL of Detection Antibody per well of assay plate.
7. Seal assay plate and incubate for 1 hour at 25 °C on microplate incubator/shaker.

1 hour 25 °C

8. Perform Post-Detection Wash.
9. Perform Post-Detection Shake for 2 minutes on microplate incubator/shaker.
10. Perform Final Aspiration.
11. Remove from washer magnet and add 10 µL of Elution Buffer B to each well of assay plate.
12. Seal and incubate for 10 minutes at 25 °C on microplate incubator/shaker.

10 minutes at 25 °C

13. Add 10 µL Buffer D to fresh 96-well plate.
14. Transfer 10 µL of eluate from assay plate to fresh 96-well plate.
15. Transfer 20 µL of neutralized eluate to SMC® read plate.
16. Seal SMC® Read Plate with aluminum adhesive plate seal for SMCxPRO® Immunoassay System.
17. Load on SMCxPRO® Immunoassay System.

Assay Characteristics

Sensitivity

Assay sensitivity measures the true limit of quantitation of an analyte and is often defined by the Lower Limit of Quantification (LLOQ). LLOQ is calculated as the lowest concentration that can achieve CVs of < 20% and the percent recovery of the standard point is still between 80%-120%. The LLOQ of IL-17A is 0.07 pg/mL. Please note that the published LLOQ is data generated during kit verification and can have minor variation between kit lots. For lot specific LLOQ data, please see the certificate of analysis.

Precision

The assay variations of SMC® Human IL-17A High Sensitivity Immunoassay Kit were studied using three different concentrations of IL-17A (generated from serial dilutions of the SMC® IL-17A standard).

The mean intra-assay variation (n=20) was calculated from a single assay for each of the three concentrations of IL-17A standard. Mean intra-assay variation was < 10%.

The mean inter-assay variation (n=6) was calculated from six independent assays of the same three dilutions of IL-17A used to determine intra-assay variation. Mean inter-assay variation was < 15%.

Specificity/Cross-Reactivity

Mouse, Rat, Equine, Canine, and Rabbit samples are detectable.

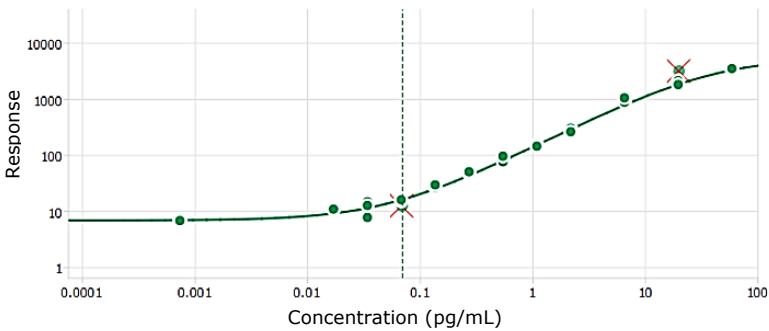
Spike Recovery

The data represent mean percent recovery of three different concentrations of standard spiked into samples (n=3 plasma samples, 2 serum samples).

Sample ID	Recovery %
Sample 1	87
Sample 2	92
Sample 3	85
Sample 4	88
Sample 5	92
Average	**89**

Graph of Typical Reference Curve

Typical SMCxPRO® Human IL-17A Immunoassay Standard Curve, not to be used to calculate data.



Troubleshooting

Problem	Probable Cause	Solution
Background is too high	Background wells were contaminated	Avoid cross-well contamination by using seal appropriately. Pipette with multichannel pipets without touching reagent in plate. Change tips when adding reagents if cross contamination is expected.
		Ensure reagents (including Wash Buffer) are not contaminated.
		Insufficient washes—washer may need to be cleaned or reprogrammed.
	Plate was over-incubated	Confirm plate incubation times are as recommended, particularly for the Detection incubation.
Sample variability is high	Multichannel pipet may not be calibrated	Calibrate pipets.
	Plate washing was not uniform	Confirm that there is no residual left in the wells following post-capture wash step and Final Aspirate. Ensure that you have < 2 µL or residual remaining in the well.
	Samples may have high particulate matter or other interfering substances	Samples should be filtered according to the Assay Preparation section. Unprocessed samples could lead to higher imprecision.
	Plate agitation was insufficient	Plate should be agitated during all incubation steps using an orbital plate shaker at a speed where beads are in constant motion without causing splashing (See Jitterbug™ Shaker setting in Assay Best Practices section).
	Cross-well contamination	Ensure that the plate is sealed well at each incubation step. If splashing occurs on plate seal, centrifuge plate at 1,100 x *g* for 1 minute to remove material prior to removing the seal. A new plate seal should be used every time the plate is sealed.
		Care should be taken when using same pipet tips that are used for reagent additions and that pipet tip does not touch reagent in plate.

Problem	Probable Cause	Solution
Beads are lost during the wash.	Plate washer needs optimization/cleaning	Contact Tech Support or local Specialist to schedule washer programming. Refer to user guide for cleaning procedure.
	Insufficiently primed washer	Washer should be primed with wash buffer prior to running the post capture wash protocol.
	Beads came in contact with water	Washer should be primed with Wash Buffer sufficiently prior to plate wash. Viscosity of water changes the performance of the magnetic particles.
	Proper magnet was not used	Ensure that the SMC® magnetic plate shipped with the BioTek® 405 TSUVS Plate Washer was present on plate wash stage prior to running wash protocol.
Published LLoQ was not achieved	Improper dilution/reconstitution of the standard reference material	Confirm appropriate kit protocol was followed when preparing standard curve.
		Check plate washer to confirm no beads were lost during washes and that plate contains < 2 µL following the post-capture and final aspiration protocols.
		Ensure standards are prepared before starting capture incubation.
Microparticles do not resuspend into homogenous solution	Beads were not properly stored and may have been frozen	Labelled microparticles should be stored at 4 °C. If microparticles are frozen, they will not resuspend properly.
	Samples may be causing interference due to excess particulate matter	Samples should be properly processed prior to testing to remove particulate matter or lipids.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7	Standard 8	Standard 9	Standard 10	Standard 11	Standard 12
B	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7	Standard 8	Standard 9	Standard 10	Standard 11	Standard 12
C	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7	Standard 8	Standard 9	Standard 10	Standard 11	Standard 12
D	Sample 1	Sample 2	Etc.									
E	Sample 1	Sample 2	Etc.									
F												
G												
H												

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