ProteinChip® Antibody Capture Kit

Instruction Manual

Catalog #K100-0005

For technical support,
call your local Bio-Rad office, or
in the US, call **1-800-4BIORAD**
(1-800-424-6723).

BIO-RAD

Introduction

Traditional immunoassay-based protein detection methods are often difficult to develop and are complicated by antibodies cross-reacting and binding nonspecifically to a variety of proteins and peptides. The ProteinChip antibody (Ab) capture kit enables unambiguous species-specific protein identification and epitope discovery experiments on one platform, and eliminates the need to develop a sandwich assay. Using the components provided in the kit, antibodies are bound to a ProteinChip array to detect antigen(s) of interest from crude samples derived from in vivo or in vitro experimental models. Once an antibody-antigen complex has been formed on the ProteinChip array, researchers can use the ProteinChip array for two unique purposes: 1) to identify proteins bound to the antibody on the basis of molecular weight using the ProteinChip SELDI reader, or 2) to conduct epitope discovery experiments by proteolytically cleaving proteins captured on ProteinChip arrays (on-chip proteolysis) and then using SELDI to highlight the peptide(s) associated with the epitope, streamlining the process of sequencing the protein binding site via tandem mass spectrometry (MS/MS).

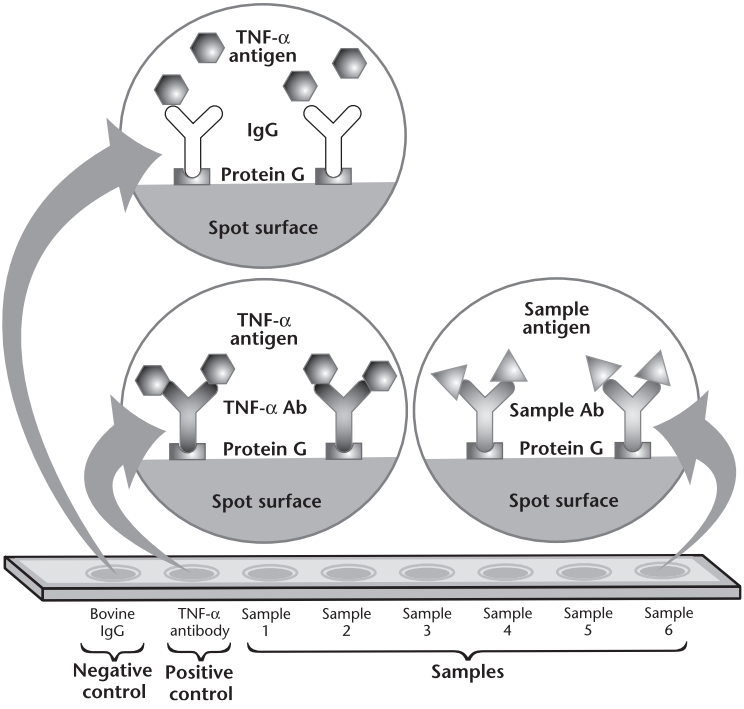


Fig. 1. Principles of the ProteinChip antibody capture kit. Protein G is covalently bound to the ProteinChip PG20 array surface. An optional crosslinking step can be performed to crosslink IgG antibodies to protein G.

The key component of this kit is the ProteinChip PG20 array (ProteinChip PS20 array precoupled with recombinant protein G (Pierce). Any antibody capable of binding protein G can be used with the kit (see appendix for details). The kit also contains positive (TNF-α antigen-antibody pair) and negative (bovine IgG) controls and a crosslinking reagent to bind antibodies covalently to ProteinChip PG20 arrays.

Materials

Materials Included

- TNF-α antibody (0.2 mg/ml anti-human TNF-α goat polyclonal antibody in phosphate buffered saline (PBS), 20 µl
- TNF-α antigen* (1.0 µM recombinant human TNF-α antigen in 0.1% bovine serum albumin (BSA)/PBS), 10 µl
- Bovine IgG (lyophilized), 750 µg
- Wash buffer (0.5% Triton X-100 in PBS); 2 bottles, 120 ml each
- PBS; 2 bottles, 120 ml each
- 1x PBS; 1 bottle, 4 ml**
- ProteinChip PG20 arrays, 6
- Bis(sulfosuccinimidyl) suberate (BS^3) crosslinking reagent (0.5 mg/vial), 2
- Deactivation buffer (0.5 M ethanolamine in PBS, pH 8.0), 4 ml
- ProteinChip sinapinic acid (SPA) energy absorbing molecules (EAMs), 5 mg
- Instruction manual

* Contains equimolar amounts of TNF with and without N-terminal methionine, yielding a double peak.

** The 4 ml PBS should be used to dilute the bovine IgG, antibody, and antigen, reconstitute the crosslinking reagent, and deposit to the spots of the ProteinChip array when needed.

Materials Needed but Not Included

- 15 ml conical tubes
- Rocking platform
- Humidity chamber*
- Lab timer
- Ice bucket and ice
- Microcentrifuge tubes
- Calibrated pipets**
- 99+% trifluoroacetic acid (TFA)

Optional Materials

- ProteinChip cassette-compatible bioprocessor (catalog #C50-30011)
- MicroMix 5 plate and tube shaker*** (Diagnostic Products Corporation)
- Sonicating water bath

Shipping and Storage

Table 1. Shipping and storage conditions for ProteinChip antibody capture kit components.

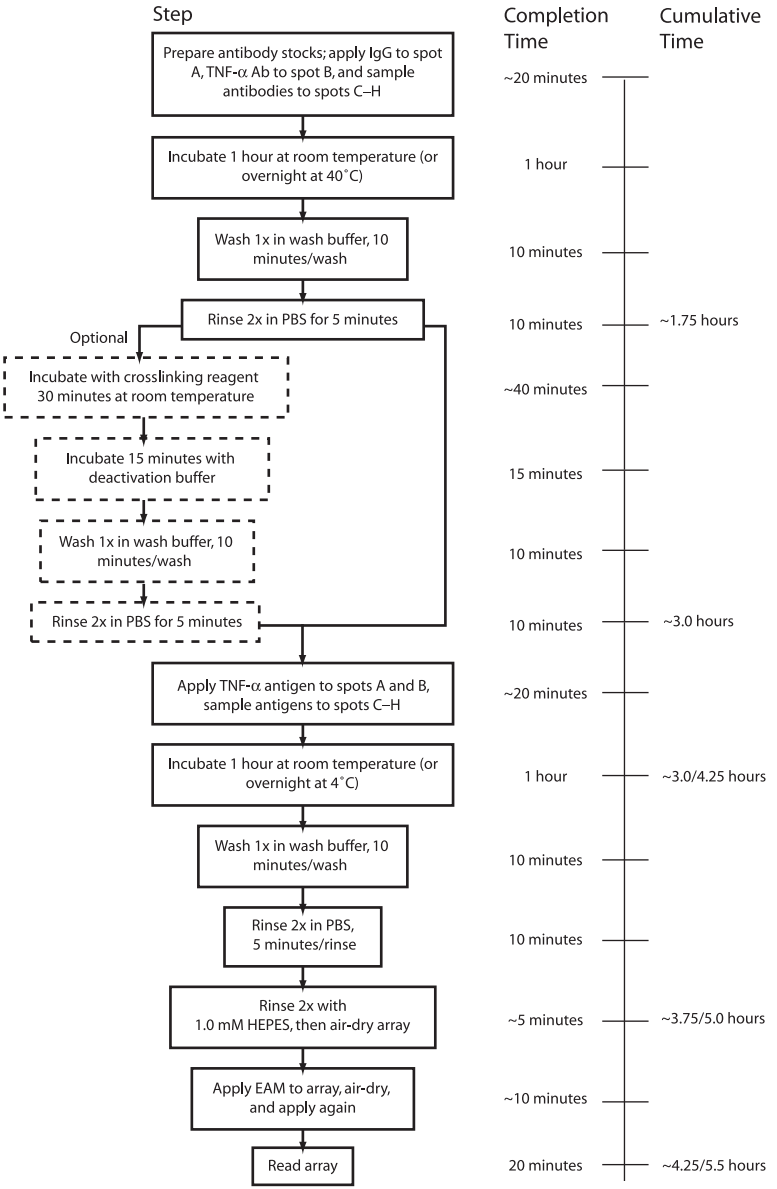
Item	Shipping	Storage
Anti-human TNF-α antibody	Dry ice	–20°C (stable 6 months) 4°C (stable 2 weeks)
Recombinant human TNF-α antigen	Dry ice	–20°C (stable 6 months) 4°C (stable 2 weeks)
Bovine IgG	Dry ice	–20°C
Crosslinking reagent	Dry ice	–20°C
ProteinChip PG20 arrays	Ambient with desiccant	4°C with desiccant
Wash buffer	Ambient	4°C
PBS	Ambient	4°C
Deactivation buffer	Ambient	4°C
HEPES buffer	Ambient	4°C
ProteinChip SPA EAMs	Ambient	Ambient or 4°C

* See the ProteinChip SELDI System Applications Guide for detailed instructions on how to create a humidity chamber if one is not readily available.

** Calibrated pipets will yield more reproducible results.

*** When using a ProteinChip bioprocessor, a MicroMix 5 is recommended.

Protocol Flowchart



Step 1: Coupling Antibody to the ProteinChip Array

1. Thaw the TNF-α antibody and TNF-α antigen on ice.
2. Reconstitute 750 µg of the bovine IgG provided in the kit with 100 µl PBS to obtain a final concentration of 7.5 mg/ml.
3. Add 4 µl reconstituted control bovine IgG to 146 µl of PBS to obtain a final concentration of 0.2 mg/ml.
4. Place a ProteinChip PG20 array on a clean, flat surface.
5. Add 2 µl of the diluted bovine IgG to spot A on the array.
6. Add 2 µl of the TNF-α antibody to spot B on the array.
7. Add 2 µl 0.2 mg/ml antibody of interest to the remaining spots on the array.
8. Immediately transfer the array to a humidity chamber and incubate for 1 hour at room temperature or overnight at 4°C. If larger sample volumes are being used with a ProteinChip bioprocessor, incubate on a rocking platform.
9. Remove the bovine IgG and the TNF-α antibody from the array using a pipet to prevent cross-contamination during the following wash steps. Do not touch the surface of the array with the pipet tip.
10. Place the entire array into a 15 ml conical tube containing 8 ml wash buffer and agitate on a rocking platform for 10 minutes at room temperature.
11. Pour off the wash buffer and add 8 ml PBS to the tube and agitate on a rocking platform for 5 minutes at room temperature.
12. Pour off the PBS and repeat step 1.11 once for a total of two washes.
13. After the PBS washes, gently blot the excess buffer from the array surface using a lint-free lab wipe without touching the active spots on the array.

Step 2: Crosslinking Antibody to Protein G (Optional)

1. Reconstitute the crosslinking reagent as follows: Warm the crosslinking reagent to room temperature, dissolve 0.5 mg (one vial) of crosslinking reagent by adding 1 ml of PBS into the vial, and vigorously vortex the vial.
2. After removing the buffer from the array (step 1.13), air-dry the array for approximately 5 minutes. Do not allow the spots to dry completely. If the spots dry, add 1 µl PBS to each spot on the array.
3. Add 1 µl of crosslinking reagent to each spot on the array.
4. Immediately transfer the array to a humidity chamber and incubate for 30 minutes at room temperature.
5. Add 1 µl deactivation buffer to each spot, transfer the array to the humidity chamber, and incubate with agitation for 15 minutes at room temperature.
6. Blot the excess buffer from the surface of the array using a lint-free lab wipe without touching the active spots on the array.

7. Place the entire array in a 15 ml conical tube containing 8 ml of wash buffer. Agitate on a rocking platform for 10 minutes. Pour off the wash buffer.
8. Pour off the wash buffer and add 8 ml of PBS to the conical tube. Agitate vigorously on a shaker for 5 minutes.
9. Repeat step 2.8 for a total of two PBS washes.

Step 3: Capturing Antigens on the ProteinChip PG20 Array

1. After completing the PBS washes, pour off the buffer. Using a lint-free lab wipe, gently blot away any excess buffer from the surface of the array without touching the active spots on the array.
2. Dilute 1 µl of TNF-α antigen into 19 µl of PBS, and vortex. The final concentration will be 50 fmol/µl.
3. Add 2 µl (100 fmol) of the diluted TNF-α antigen to spots A and B on the array.
4. Add 2 µl of sample to the remaining spots on the array (C–H), and transfer the array to a humidity chamber.
5. Incubate the array for 1 hour at room temperature, or incubate overnight at 4°C.
6. Place the array in a 15 ml conical tube containing 8 ml of wash buffer. Agitate on a rocking platform for 10 minutes. Pour off the wash buffer.
7. Add 8 ml of PBS to the conical tube. Agitate on a rocking platform for 5 minutes at room temperature.
8. Pour off the PBS, add 8 ml fresh PBS, and agitate for 5 minutes.
9. Make 500 ml of a 1 mM HEPES solution by adding 500 µl of 1 M HEPES to 500 ml deionized water.
10. Pour off the PBS and rinse the array two times with 1 mM HEPES.
11. Remove the array from the conical tube, flick off the HEPES, and allow to air-dry for approximately 10 minutes.

Step 4: EAM Preparation and Addition

1. Add 100 µl 99.8% acetonitrile and 100 µl 1.0% TFA into the vial containing ProteinChip SPA EAM powder to obtain a final concentration of 25 mg/ml.
2. Vortex the vial for 5 minutes to dissolve the EAM powder. Alternatively, incubate the vial for 5 minutes in a sonicating water bath.
3. Add 0.5 µl of this saturated EAM solution to each spot on the array and air-dry.
4. Repeat step 4.3 and air-dry the array completely (approximately 10 minutes) before reading in a ProteinChip SELDI reader.

Step 5: Reading the ProteinChip PG20 Array in a ProteinChip SELDI Reader

After the EAM has dried on the array, read the array in a ProteinChip SELDI reader. The spot protocol used to read the array can be created once and saved. The data collection parameters of the spot protocol are a function of molecular mass and need to be adjusted for each antigen. See the operation manual for ProteinChip data manager software for detailed instructions on how to create an optimized spot protocol. When optimizing the protocol for the detection of TNF-α antigen (molecular mass 17.5 kD), set the Mass Range limits from 0 to 200,000 Da, and the Focus Mass to 17,500 Da.

Ordering Information

Catalog #	Description
K10-00005	**ProteinChip Antibody Capture Kit**, includes antibody, antigen, reagents, controls, 6 ProteinChip PG20 arrays, ProteinChip SPA energy absorbing molecules (EAMs), instructions
C55-30058	**ProteinChip PG20 Array**, A–H format
C50-30011	**ProteinChip Cassette-Compatible Bioprocessor**, includes ProteinChip array forceps, cassette hold-down frame, 12 blank ProteinChip arrays
C30-00002	**ProteinChip SPA Energy Absorbing Molecules (EAMs)**, 5 mg/vial, 20

Appendix A

Immunoglobulin Binding Affinity to Protein G

The information in Table 2 is provided as a guideline and does not guarantee that all antibodies will behave identically to the information provided in the table.

Table 2: Binding affinity of various immunoglobulins to protein G.*

Species	Immunoglobulin	Binding Affinity**
Human	Total IgG	S
	IgG_1	S
	IgG_2	S
	IgG_3	S
	IgG_4	S
Mouse	Total IgG	S
	IgG_1	M
	IgG_{2a}	S
	IgG_{2b}	S
	IgG_3	S
Rat	Total IgG	M
	IgG_1	M
	IgG_{2a}	S
	IgG_{2b}	W
	IgG_{2c}	S
Bovine	Total IgG	S
	IgG1	S
	IgG2	S
Cat	Total IgG	W
Dog	Total IgG	W
Goat	Total IgG	S
	IgG_1	S
	IgG_2	S
Guinea pig	Total IgG	W
Horse	Total IgG	S
	$IgG_{(ab)}$	NB
	$IgG_{(c)}$	MB
	IgG(T)	S
Pig	Total IgG	W
Rabbit	Total IgG	S
Sheep	Total IgG	S
	IgG_1	S
	IgG_2	S

* Information provided by Pierce.

** W, weak binding; M, medium binding; S, strong binding; NB, no binding.

Spectra of TNF-α Antigen Capture

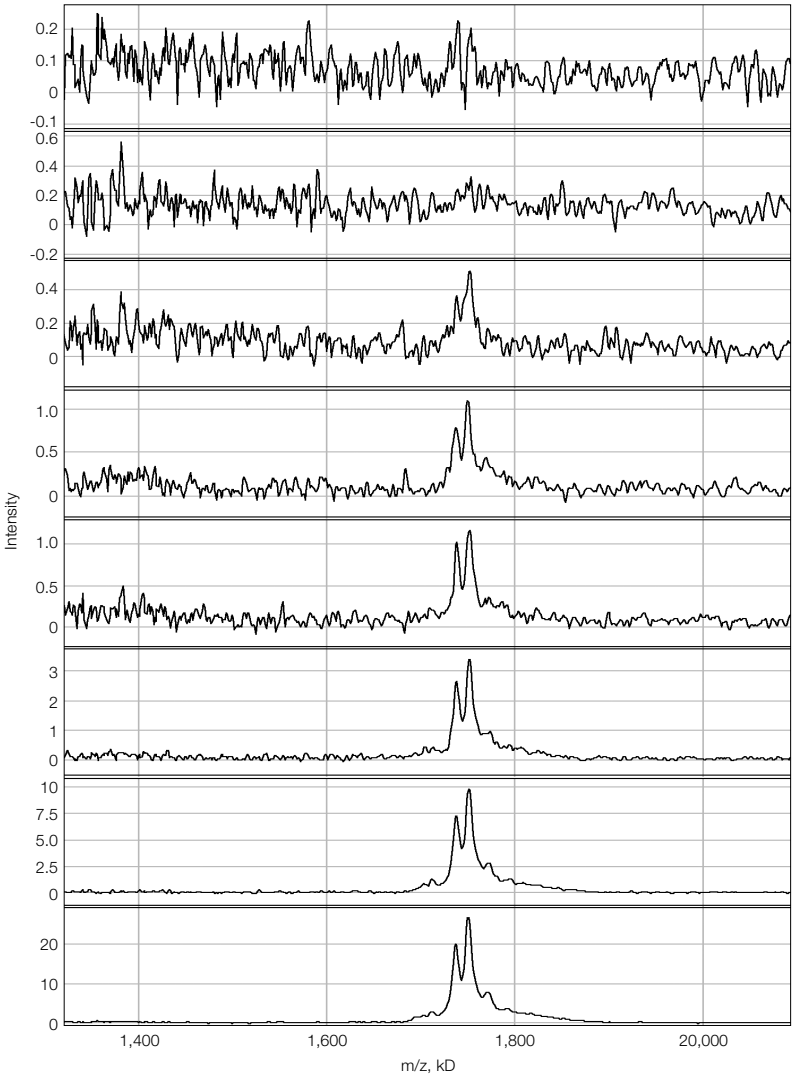


Fig. 2. Spectra illustrating the effect of increasing TNF-a quantity on intensity. The molecular mass of TNF-α is 17.5 kD. TNF-α quantities range from 130 amol to 532 fmol. The double peak in these spectra represents equimolar amounts of TNF-a with and without N-terminus methionine.

Covalently Crosslinking IgG Antibodies to Protein G

Antibodies captured by protein G on ProteinChip PG20 arrays are not covalently bound to the surface of the array. When these arrays are analyzed in a ProteinChip SELDI reader, peaks may be seen in the resulting spectra corresponding to intact IgG (MH+ ~148 kD, M2H+ ~75 kD) or its fragments (~75 kD, ~48 kD, ~22 kD), especially if a high laser energy is used. These peaks may interfere with the detection or quantitation of an antigen of similar molecular weight. When the antibody is crosslinked to protein G on the array, it becomes covalently attached to the array surface and will not be desorbed from the array surface during the reading process.

The results of an antibody capture assay depicting crosslinked and noncrosslinked antibody are shown in Figure 3. Without crosslinking, an IgG peak (molecular mass 148 kD) is often seen. When the IgG is crosslinked to the ProteinChip PG20 array, the IgG peak is not observed. Crosslinking does not affect the peak intensity of TNF-α antigen.

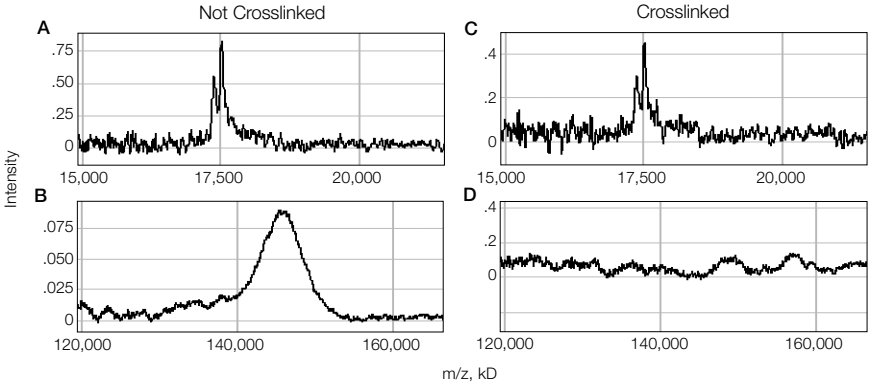


Fig. 3. Effect of crosslinking on TNF-α antigen and TNF-α antibody peak profiles. A, peak corresponding to the 17.5 kD protein, TNF-α, is seen when 100 fmol TNF-α is applied to a ProteinChip PG20 array; B, peak corresponding to the 145 kD TNF-α IgG antibody is seen when 2.7 pmol of antibody is applied to a ProteinChip PG20 array; C, peak corresponding to the 17.5 kD protein, TNF-α, is seen when 100 fmol TNF-α is applied to a ProteinChip PG20 array and the anti-TNF-α antibody is crosslinked to the array; D, no peak is seen corresponding to the 145 kD TNF-α IgG antibody when 2.7 pmol of antibody is added to a ProteinChip PG20 array.

Performance Specifications

Using the reagents supplied with the ProteinChip antibody capture kit, the user should be able to detect the TNF-α antigen positive control (1.0 µM recombinant human TNF-α antigen in 0.1% BSA/PBS) down to a level of 1 fmol at a signal-to-noise ratio of at least 3. For the TNF-α control, the quantitation range will generally be from 10–500 fmol.

Different antigens behave significantly differently in the antibody capture and SELDI processes, so these values cannot be applied to any other antigen-antibody system. The lower limit of detection and dynamic range for each antigen needs to be determined for each system.

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 4BIORAD* ***Australia*** *61 02 9914 2800*
Austria *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 21 3237 9400*
Canada *905 712 2771* ***China*** *86 21 6426 0808* ***Czech Republic*** *420 241 430 532*
Denmark *44 52 10 00* ***Finland*** *09 804 22 00* ***France*** *01 47 95 69 65*
Germany *089 318 84 0* ***Greece*** *30 210 777 4396* ***Hong Kong*** *852 2789 3300*
Hungary *36 1 455 8800* ***India*** *91 124 4029300* ***Israel*** *03 963 6050*
Italy *39 02 216091* ***Japan*** *03 5811 6270* ***Korea*** *82 2 3473 4460*
Mexico *52 555 488 7670* ***The Netherlands*** *0318 540666* ***New Zealand*** *0508 805 500*
Norway *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700*
Russia *7 495 721 14 04* ***Singapore*** *65 6415 3188* ***South Africa*** *27 861 246 723*
Spain *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *061 717 95 55*
Taiwan *886 2 2578 7189* ***United Kingdom*** *020 8328 2000*

10008252 US/EG Rev D *06-0733 0307 Sig 1106*