

Claims

1. A method of determining an amount of free IL-17 in a sample comprising IL-17 and a small molecule (SM) modulator of IL-17, the method comprising:
 - i. contacting the sample with a first capture antibody to form a mixture comprising a first complex comprising the first capture antibody and the IL-17 unbound to the SM modulator, and a second complex comprising the first capture antibody and the IL-17 bound to the SM modulator;
 - ii. contacting the mixture from step i) with a first detection agent to thereby form a third complex comprising the first capture antibody, the IL-17 unbound to the SM modulator, and the first detection agent, but not a fourth complex comprising the first capture antibody, the IL-17 bound to the SM modulator, and the first detection agent; and
 - iii. determining the amount of the free IL-17 by measuring the amount of the first detection agent in the third complex,wherein the SM modulator of IL-17 is a compound that can bind to IL-17 and has a molecular weight ranging from about 100 g/mol; to about 1500 g/mol, preferably, the first detection agent is a first detection antibody.
2. The method of claim 1, wherein no wash step is performed before contacting the mixture from step i) with the first detection agent.
3. A method of determining the target engagement of a SM modulator of IL-17 in a subject, comprising a) determining the amount of free IL-17 in a sample from the subject before administration of the SM modulator using the method of claim 1; b) determining the amount of free IL-17 in a sample from the subject after administration of the SM modulator using the method of claim 1; and c) comparing the amounts of free IL-17 determined from steps a) and b).
4. A method of determining the target engagement of a SM modulator of IL-17 in a subject, comprising a) determining the amount of free IL-17 in a sample from the subject at two or more time points after the administration of the SM modulator using the method of claim 1; and b) comparing the amounts of free IL-17 at the two or more time points determined from step a).
5. A method of determining an amount of modulator-bound IL-17 in a sample comprising

IL-17 and a SM modulator of IL-17, the method comprising:

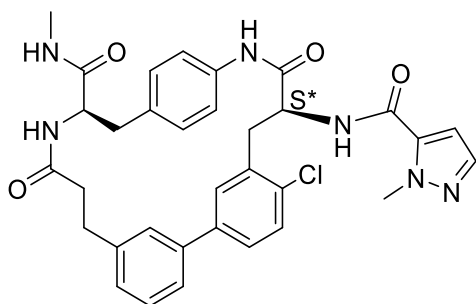
- i. contacting a first portion of the sample with a first capture antibody to form a first mixture comprising a first complex comprising the first capture antibody and the IL-17 unbound to the SM modulator, and a second complex comprising the first capture antibody and the IL-17 bound to the SM modulator;
- ii. contacting the first mixture from step i) with a first detection agent to thereby form a third complex comprising the first capture antibody, the IL-17 unbound to the SM modulator, and the first detection agent, but not a fourth complex comprising the first capture antibody, the IL-17 bound to the SM modulator, and the first detection agent;
- iii. contacting a second portion of the sample with a second capture antibody to form a second mixture comprising a fifth complex comprising the second capture antibody and the IL-17 unbound to the SM modulator, and a sixth complex comprising the second capture antibody and the IL-17 bound to the SM modulator;
- iv. contacting the second mixture with a second detection agent to thereby form a seventh complex comprising the second capture antibody, the IL-17 unbound to the SM modulator, and the second detection agent and an eighth complex comprising the second capture antibody, the IL-17 bound to the SM modulator, and the second detection agent;
- v. determining the amount of the free IL-17 by measuring the amount of the first detection agent in the third complex;
- vi. determining a total amount of the IL-17 by measuring the total amount of the second detection agent in the seventh complex and the eighth complex; and
- vii. determining the amount of the modulator-bound IL-17 in the sample by subtracting the amount of the free IL-17 determined in step (v) from the total amount of the IL-17 in step (vi),

wherein the SM modulator of IL-17 is a compound that can bind to IL-17 and has a molecular weight ranging from about 100 g/mol; to about 1500 g/mol, preferably, the first detection agent is a first detection antibody and the second detection agent is a second detection antibody.

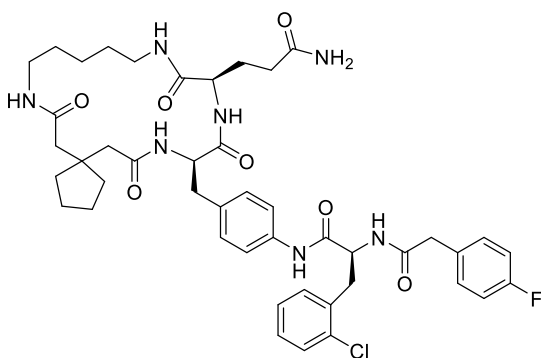
6. A method of determining the target engagement of a SM modulator of IL-17 in a subject,

comprising determining the amount of modulator-bound IL-17 in a sample from the subject after administration of the SM modulator using the method of claim 5.

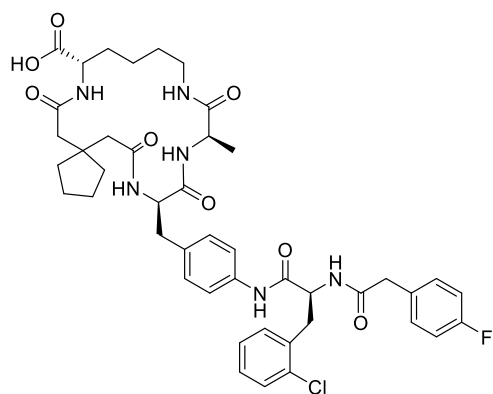
7. The method of any one of claims 1-6, wherein the SM modulator has a molecular weight of about 100-1500 g/mol.
8. The method of claim 7, wherein the SM modulator has a molecular weight of about 400-1050 g/mol, or about 500-1000 g/mol, or about 600-900 g/mol.
9. The method of claim 7, wherein the SM modulator has a molecular weight of about 300-750 g/mol, or about 500-800 g/mol, or about 600-900 g/mol.
10. The method of any one of claims 1 to 6, wherein the SM modulator is selected from the group consisting of Compounds #1-6 having the following structures and pharmaceutically acceptable salts thereof:



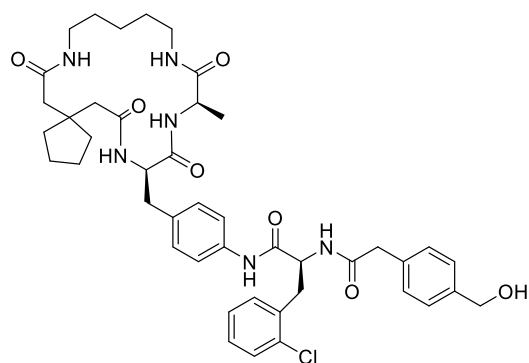
(Compound #1)



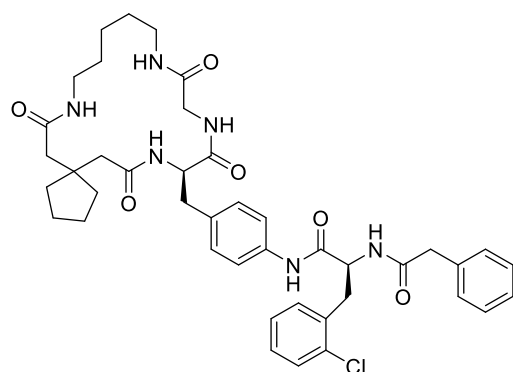
(Compound #2)



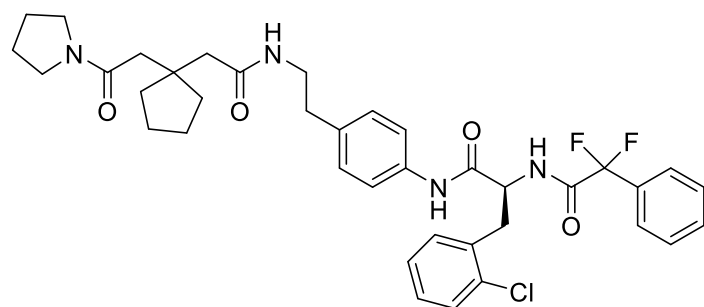
(Compound #3)



(Compound #4)

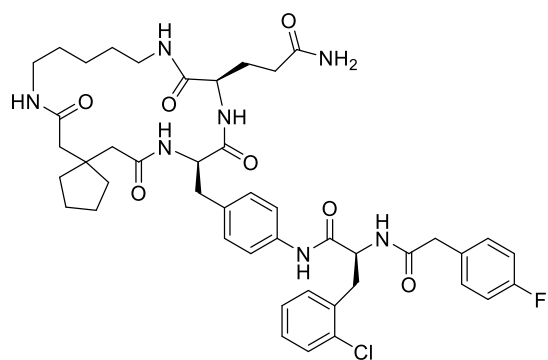


(Compound #5)

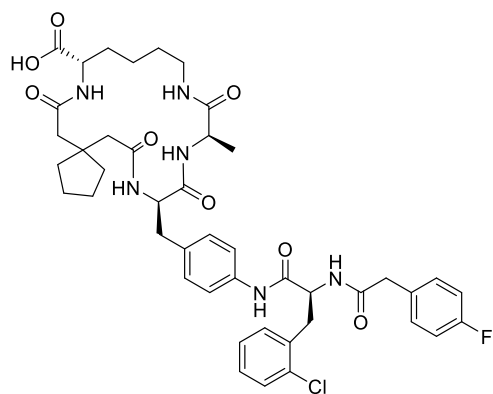


(Compound #6).

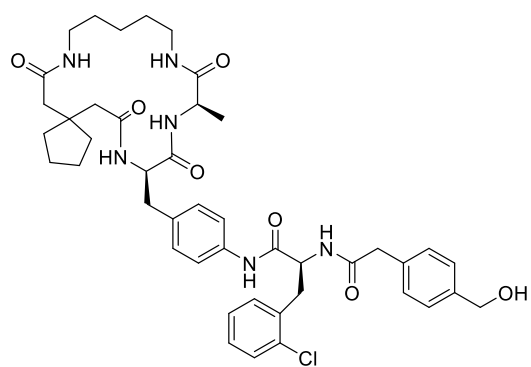
11. The method of any one of claims 1 to 6, wherein the SM modulator is selected from the group consisting of Compounds #2-6 having the following structures and pharmaceutically acceptable salts thereof:



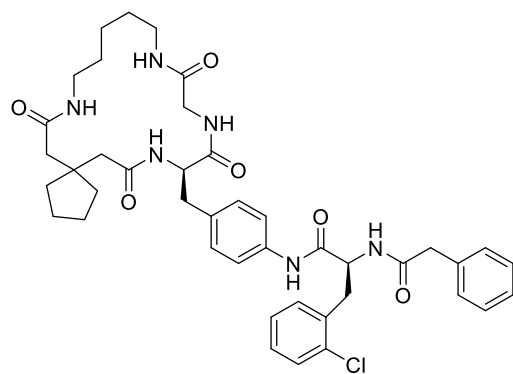
(Compound #2)



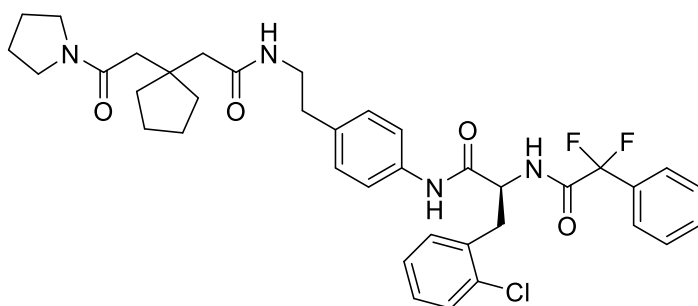
(Compound #3)



(Compound #4)



(Compound #5)



(Compound #6).

12. The method of any one of claims 1-11, wherein the first and/or the second detection agent is labeled with a detectable label, more preferably, the label is an enzyme or biotin.
13. The method of any one of claims 1-12, wherein the first capture antibody and the first detection agent are selected from the pairs in the following table:

First capture antibody		First detection agent	
Company	CAT# or ID	Company	CAT# or ID
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Thermo Fisher Scientific, Inc.	Detection antibody of HS ELISA human IL-17A Kit BMS2017HS
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	NOVUS Biologicals LLC	Detection antibody of HS ELISA human IL17A Kit #NBP1-82423
Janssen Biotech, Inc.	mAb7024	NOVUS Biologicals LLC	Detection antibody of HS ELISA human IL17A Kit #NBP1-82423
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	BioLegend, Inc.	Anti-human IL-17A antibody 512702
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Janssen Biotech, Inc.	mAb3584
Quanterix Corp.	Capture antibody of IL-17A Advantage Kit 101599	Janssen Biotech, Inc.	mAb3584
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Janssen Biotech, Inc.	mAb3077
Quanterix Corp.	Capture antibody of IL-17A Advantage Kit 101599	Janssen Biotech, Inc.	mAb3077
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Janssen Biotech, Inc.	mAb732
Quanterix Corp.	Capture antibody of IL-17A Advantage Kit 101599	Janssen Biotech, Inc.	mAb732
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Janssen Biotech, Inc.	mAb7024
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Janssen Biotech, Inc.	mAb4538
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Janssen Biotech, Inc.	mAb5548

R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Creative Diagnostics, Inc.	Anti-IL 17A monoclonal antibody DCABH-4543
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Creative Diagnostics, Inc.	Anti-IL 17A monoclonal antibody CABT-ZC1183
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Creative Diagnostics, Inc.	Anti-IL 17A monoclonal antibody CABT-WN1669
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Invitrogen	IL-17A antibody MA1069B
Quanterix Corp.	Capture antibody of IL-17A Advantage Kit 101599	Invitrogen	IL-17A antibody MA1069B
Janssen Biotech, Inc.,	mAb7024	Invitrogen	IL-17A antibody MA1069B
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Mabtech AB	Anti-human IL-17A antibody 3520-5N-500
Janssen Biotech, Inc.	mAb7024	Mabtech AB	Anti-human IL-17A antibody 3520-5N-500
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Sino Biological Inc.	Antibody ABIN5507411
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	R&D Systems, Inc.	Recombinant Human IL-17RA/IL-17R Fc Chimera Protein, CF, 177-IR-100
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Sino Biological Inc.	IL-17RA protein 10895-H08H-B

14. The method of claim 13, wherein the first capture antibody is capture antibody of DuoSet ELISA kit DY317 (from R&D Systems, Inc.) and the first detection agent is human mAb4538 antibody (from Janssen Biotech, Inc.).
15. The method of any one of claims 5-14, wherein the second capture antibody is Capture antibody of DuoSet ELISA kit DY317 and the second detection agent are selected from the pairs in the following table:

Second capture antibody		Second detection agent	
Company	CAT#	Company	CAT#
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	R&D Systems, Inc.	Detection antibody of DuoSet ELISA kit DY317
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	R&D Systems, Inc.	Detection antibody of human IL-17 HS ELISA Kit HS170
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	CovalAb R&D (Antibodies Online)	antibody ABIN1724556
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Creative Diagnostics	Anti-IL 17A monoclonal antibody CABT-ZC1184
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Mabtech AB	Anti-human IL-17 antibody 3520-3-250

R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	MyBioSource (MBS)	Anti-IL-17A antibody MBS592444
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	MyBioSource (MBS)	Anti-IL-17A antibody MBS592430
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	MyBioSource (MBS)	Anti-IL-17A antibody MBS689609
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	MyBioSource (MBS)	Anti-IL-17A antibody MBS592432
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	NOVUS Biologicals LLC	IL-17/IL-17A antibody DDX0336B-100
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Antibodies Online	ABIN786722
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	OriGene Technologies Inc.	DDX0334B-50
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	OriGene Technologies Inc.	DDX0335B-50
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Rockland Immunochemicals Inc.	IL-17A biotin conjugated antibody 209-306-B32

16. The method of claim 15, wherein the second capture antibody and the second detection agent are capture antibody and detection antibody of DuoSet ELISA kit DY317 (from R&D Systems, Inc.), respectively.
17. The method of any one of claims 1-16, wherein the sample is a biological sample obtained from a subject under an *ex vivo* or *in vivo* treatment with the SM modulator.
18. The method of claim 17, wherein the subject is a mammal, preferably human.
19. The method of claim 17 or 18, wherein the biological sample is selected from the group consisting of a sample prepared from cells, tissues, or a serum, plasma or another biological fluid sample.
20. The method of any one of claims 1-19, wherein the method has a detection sensitivity with Lower Limit of Quantification (LLOQ) at about 0.01 or about 0.1 or about 0.25, or about 0.5, or about 0.75, or about 1 pg/ml of the IL-17 in the sample.
21. A kit for determining an amount of free IL-17 in a sample comprising IL-17 and a SM modulator of IL-17, the kit comprising:
 - i. a first capture antibody, wherein upon contact with the sample, the first capture antibody forms a first complex comprising the first capture antibody and the IL-17 unbound to the SM modulator, and a second complex comprising the first capture

- antibody and the IL-17 bound to the SM modulator; and
- ii. a first detection agent, wherein upon contact with the first complex and the second complex, the first detection agent, forms a third complex comprising the first capture antibody, the IL-17 unbound to the SM modulator, and the first detection agent, but not a fourth complex comprising the first capture antibody, the IL-17 bound to the SM modulator, and the first detection agent,
- wherein the SM modulator of IL-17 is a compound that can bind to IL-17 and has a molecular weight ranging from about 100 g/mol; to about 1500 g/mol, preferably, the first detection agent is a first detection antibody.
22. A kit for determining an amount of modulator-bound IL-17 in a sample comprising IL-17 and a SM modulator of IL-17, the kit comprising:
- i. a first capture antibody, wherein upon contact with the sample, the first capture antibody forms a first complex comprising the first capture antibody and the IL-17 unbound to the SM modulator, and a second complex comprising the first capture antibody and the IL-17 bound to the SM modulator;
 - ii. a first detection agent, wherein upon contact with the first complex and the second complex, the first detection agent, forms a third complex comprising the first capture antibody, the IL-17 unbound to the SM modulator, and the first detection agent, but not a fourth complex comprising the first capture antibody, the IL-17 bound to the SM modulator, and the first detection agent; and
 - iii. a second capture antibody, wherein upon contact with the sample, the second capture antibody forms a fifth complex comprising the second capture antibody and the IL-17 unbound to the SM modulator, and a sixth complex comprising the second capture antibody and the IL-17 bound to the SM modulator;
 - iv. a second detection agent, wherein upon contact with the fifth complex and the sixth complex, the second detection agent, forms a seventh complex comprising the second capture antibody, the IL-17 unbound to the SM modulator, and the second detection agent, and an eighth complex comprising the second capture antibody, the IL-17 bound to the SM modulator, and the second detection agent, respectively,
- wherein the SM modulator of IL-17 is a compound that can bind to IL-17 and has a molecular weight ranging from about 100 g/mol; to about 1500 g/mol,

preferably, the first detection agent is a first detection antibody and the second detection agent is a second detection antibody.

23. The kit of claim 21 or 22, wherein the first detection agent and/or the second detection agent, is labeled with a detectable label, more preferably, the label is an enzyme or biotin.
24. The kit of any one of claims 21-23, wherein the first capture antibody and the first detection agent are selected from the pairs in the following table:

First capture antibody		First detection agent	
Company	CAT# or ID	Company	CAT# or ID
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Thermo Fisher Scientific, Inc.	Detection antibody of HS ELISA human IL-17A Kit BMS2017HS
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	NOVUS Biologicals LLC	Detection antibody of HS ELISA human IL17A Kit #NBP1-82423
Janssen Biotech, Inc.	mAb7024	NOVUS Biologicals LLC	Detection antibody of HS ELISA human IL17A Kit #NBP1-82423
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	BioLegend, Inc.	Anti-human IL-17A antibody 512702
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Janssen Biotech, Inc.	mAb3584
Quanterix Corp.	Capture antibody of IL-17A Advantage Kit 101599	Janssen Biotech, Inc.	mAb3584
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Janssen Biotech, Inc.	mAb3077
Quanterix Corp.	Capture antibody of IL-17A Advantage Kit 101599	Janssen Biotech, Inc.	mAb3077
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Janssen Biotech, Inc.	mAb732
Quanterix Corp.	Capture antibody of IL-17A Advantage Kit 101599	Janssen Biotech, Inc.	mAb732
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Janssen Biotech, Inc.	mAb7024
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Janssen Biotech, Inc.	mAb4538
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Janssen Biotech, Inc.	mAb5548
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Creative Diagnostics, Inc.	Anti-IL 17A monoclonal antibody DCABH-4543
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Creative Diagnostics, Inc.	Anti-IL 17A monoclonal antibody CABT-ZC1183
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Creative Diagnostics, Inc.	Anti-IL 17A monoclonal antibody CABT-WN1669

R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Invitrogen	IL-17A antibody MA1069B
Quanterix Corp.	Capture antibody of IL-17A Advantage Kit 101599	Invitrogen	IL-17A antibody MA1069B
Janssen Biotech, Inc.,	mAb7024	Invitrogen	IL-17A antibody MA1069B
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Mabtech AB	Anti-human IL-17A antibody 3520-5N-500
Janssen Biotech, Inc.	mAb7024	Mabtech AB	Anti-human IL-17A antibody 3520-5N-500
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Sino Biological Inc.	Antibody ABIN5507411
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	R&D Systems, Inc.	Recombinant Human IL-17RA/IL-17R Fc Chimera Protein, CF, 177-IR-100
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Sino Biological Inc.	IL-17RA protein 10895-H08H-B

25. The kit of claim 24, wherein the first capture antibody is capture antibody of DuoSet ELISA kit DY317 (from R&D Systems, Inc.) and the first detection agent is human mAb4538 antibody (from Janssen Biotech, Inc.)
26. The kit of any one of claims 22-25, wherein the second capture antibody and the second detection agent are selected from the pairs in the following table:

Second capture antibody		Second detection agent	
Company	CAT#	Company	CAT#
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	R&D Systems, Inc.	Detection antibody of DuoSet ELISA kit DY317
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	R&D Systems, Inc.	Detection antibody of human IL-17 HS ELISA Kit HS170
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	CovalAb R&D (Antibodies Online)	antibody ABIN1724556
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Creative Diagnostics	Anti-IL 17A monoclonal antibody CABT-ZC1184
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Mabtech AB	Anti-human IL-17 antibody 3520-3-250
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	MyBioSource (MBS)	Anti-IL-17A antibody MBS592444
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	MyBioSource (MBS)	Anti-IL-17A antibody MBS592430
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	MyBioSource (MBS)	Anti-IL-17A antibody MBS689609

R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	MyBioSource (MBS)	Anti-IL-17A antibody MBS592432
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	NOVUS Biologicals LLC	IL-17/IL-17A antibody DDX0336B-100
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Antibodies Online	ABIN786722
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	OriGene Technologies Inc.	DDX0334B-50
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	OriGene Technologies Inc.	DDX0335B-50
R&D Systems, Inc.	Capture antibody of DuoSet ELISA kit DY317	Rockland Immunochemicals Inc.	IL-17A biotin conjugated antibody 209-306-B32

27. The kit of claim 26, wherein the second capture antibody and the second detection agent are capture antibody and detection antibody of DuoSet ELISA kit DY317 (from R&D Systems, Inc.), respectively.