Quantikine® ELISA

Mouse IL-4 Immunoassay

Catalog Number M4000B
SM4000B
PM4000B

For the quantitative determination of mouse Interleukin 4 (IL-4) concentrations in cell culture supernates and serum.

Note: The standard reconstitution method has changed. Read this package insert in its entirety before using this product.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

SECTION	PAGE
INTRODUCTION	1
PRINCIPLE OF THE ASSAY	2
LIMITATIONS OF THE PROCEDURE	2
TECHNICAL HINTS	2
MATERIALS PROVIDED & STORAGE CONDITIONS	3
OTHER SUPPLIES REQUIRED	4
PRECAUTIONS	4
SAMPLE COLLECTION & STORAGE	4
SAMPLE PREPARATION	4
REAGENT PREPARATION	5
ASSAY PROCEDURE	6
CALCULATION OF RESULTS	7
TYPICAL DATA	7
PRECISION	8
RECOVERY	8
LINEARITY	8
SENSITIVITY	9
CALIBRATION	9
SAMPLE VALUES	9
SPECIFICITY	10
REFERENCES	10

MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

Interleukin-4 (IL-4), also known as B cell-stimulatory factor-1, is an approximately 18-20 kDa monomeric cytokine that displays pleiotropic effects during immune responses (1-4). Mouse IL-4 is synthesized as a 140 amino acid (aa) precursor with a 20 aa signal sequence and a 120 aa mature chain. The cytokine contains three potential sites for N-linked glycosylation, three intrachain disulfide bonds, and adopts a bundled four α-helix structure (5). Analogous to human, mouse IL-4 has a reported alternative splicing short form. Unlike human, this form is suggested to be very minor in expression and of questionable significance (6). Mature mouse IL-4 shares 43%, and 63% aa sequence identity with human and rat IL-4, respectively. Research has shown that human, mouse, and rat IL-4 are all species-specific in their activities (7-9). IL-4 is expressed by Th2-biased $CD4^+$ T cells, basophils, mast cells, NKT and γδ T cells, and eosinophils (1-2, 10).

IL-4 is a key player in the type 2 immune response as it promotes T helper type 2 (Th2) differentiation and B cell commitment to the immunoglobulin G1 and immunoglobulin E isotypes (11). IL-4 initiates Th2 differentiation by binding to either a type I or type II receptor complex that contains the IL-4 Rα subunit coupled to either the γ chain or the IL-13Rα1, respectively (12). Phosphorylation of the cytoplasmic C-terminal tails by the Janus Kinase (JAK) family of tyrosine kinases results from the heterodimerization of the IL-4 receptor on the cell surface (12). This then leads to the recruitment and phosphorylation of STAT6 (12). Following the phosphorylation of STAT6, conformational changes take place leading to dimerization, nuclear translocation, DNA binding and transcriptional activation of several target genes including the genes for IL-4, IL-5, and IL-13, and the Th2 specific factors GATA3 and c-Maf (12).

Functionally, IL-4 promotes cell proliferation and survival, immunoglobulin class switch to IgG_1 and IgE in mouse B cells, priming and chemotaxis of mast cells, eosinophils, and basophils, an acquisition of the Th2 phenotype by naïve $CD4^+$ cells, and the proliferation and activation of epithelial cells (13-16). IL-4 is also a significant cytokine in tumor immunology (17). Researchers found in early mouse experiments that IL-4 exhibited potent anti-tumor ability. The mice rejected IL-4 producing tumors and developed long-lasting anti-tumor immunity (17). This is perhaps due to IL-4's anti-angiogenic effect and/or its ability to activate select $CD8^+$ T cells. Paradoxically, new evidence shows that IL-4 is a tumor-promoting molecule, and thus a cytokine with opposing effects (17). This is possible due to the IL-4 induced upregulation of anti-apoptotic molecules in tumor cells, and the downregulation of cytolytic molecules on $CD8^+$ T cells. In addition, tumor produced IL-4 is also suggested to act on local tumor-associated-macrophages (TAMs), inducing the secretion of cathepsins that promote cell migration (18).

The Quantikine® Mouse IL-4 Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse IL-4 levels in cell culture supernates and serum. It contains *E. coli*-expressed recombinant mouse IL-4 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant mouse IL-4 accurately. Results obtained using natural mouse IL-4 showed dose curves that were parallel to the standard curves obtained using the recombinant Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse IL-4.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IL-4 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-4 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IL-4 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of IL-4 bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- For best results, pipette reagents and samples into the center of each well.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # M4000B	CATALOG # SM4000B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse IL-4 Microplate	890408	2 plates	6 plates	96 well polystyrene microplates (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse IL-4.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse IL-4 Standard	890401	1 vial	3 vials	Recombinant mouse IL-4 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer. Avoid repeated freeze-thaw cycles.*
Mouse IL-4 Control	890242	1 vial	3 vials	Recombinant mouse IL-4 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse IL-4 Conjugate	892701	1 vial	3 vials	23 mL/vial of a polyclonal antibody specific for mouse IL-4 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-18	895202	1 vial	3 vials	12 mL/vial of a buffered protein solution with preservatives.	
Calibrator Diluent RD5Y	895201	2 vials	6 vials	21 mL/vial of a buffered protein solution with preservatives.	
Wash Buffer Concentrate	895003	2 vials	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	3 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	3 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	1 vial	3 vials	23 mL/vial of diluted hydrochloric acid.	
Plate Sealers	N/A	8 strips	24 strips	Adhesive strips.	

* Provided this is within the expiration date of the kit.

M4000B contains sufficient materials to run ELISAs on two 96 well plates.
SM4000B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PM4000B). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the literature accompanying your order for specific vial counts.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards and samples.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Grossly hemolyzed or lipemic samples may not be suitable for use in this assay.*

SAMPLE PREPARATION

Serum samples require a 3-fold dilution into Calibrator Diluent RD5Y. A suggested 3-fold dilution is 50 µL of sample + 100 µL of Calibrator Diluent RD5Y.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

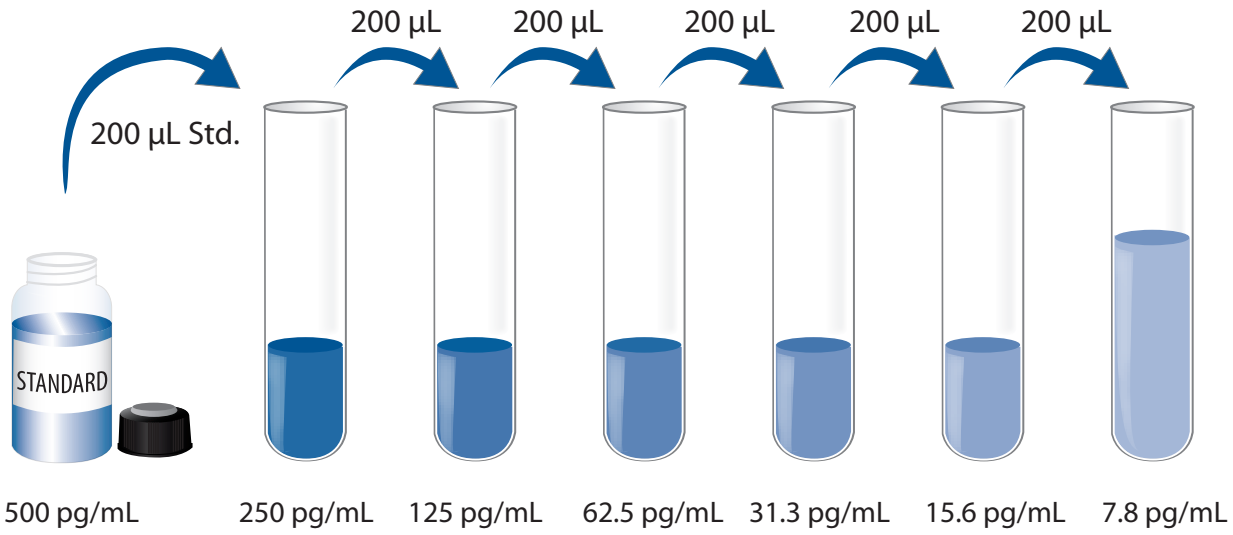
Mouse IL-4 Control - Reconstitute the control with 1.0 mL deionized or distilled water. Mix thoroughly. Assay the control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, add 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Mouse IL-4 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IL-4 Standard with Calibrator Diluent RD5Y. Do not substitute other diluents. This reconstitution produces a stock solution of 500 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5Y into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse IL-4 Standard (500 pg/mL) serves as the high standard. Calibrator Diluent RD5Y serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and control be assayed in duplicate.

1. Prepare reagents, standard dilutions, control, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1-18 to each well.
4. Add 50 µL of standard, control, or sample* per well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Mouse IL-4 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in Step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

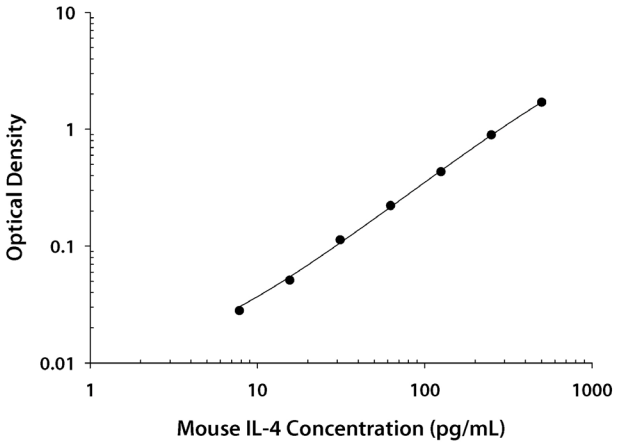
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse IL-4 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.070 0.065	0.068	—
7.8	0.094 0.097	0.096	0.028
15.6	0.120 0.118	0.119	0.051
31.3	0.179 0.183	0.181	0.113
62.5	0.293 0.287	0.290	0.222
125	0.508 0.492	0.500	0.432
250	0.960 0.962	0.961	0.893
500	1.775 1.761	1.768	1.700

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	20.1	58.6	199	20.7	58.8	203
Standard deviation	1.3	1.8	8.9	1.2	2.7	10.6
CV (%)	6.5	3.1	4.5	5.8	4.6	5.2

RECOVERY

The recovery of mouse IL-4 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=8)	95	84-109%
Serum* (n=7)	95	88-118%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with various concentrations of mouse IL-4 in each matrix were diluted with Calibrator Diluent RD5Y and then assayed.

		Cell culture supernates (n=4)	Serum (n=4)
1:2	Average % of Expected	104	108
	Range (%)	100-109	106-110
1:4	Average % of Expected	105	109
	Range (%)	100-111	106-112
1:8	Average % of Expected	105	110
	Range (%)	93-117	107-112
1:16	Average % of Expected	102	100
	Range (%)	94-113	91-108

SENSITIVITY

The minimum detectable dose (MDD) of mouse IL-4 is typically < 2 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant mouse IL-4 produced at R&D Systems®.

The NIBSC/WHO interim reference mouse IL-4 preparation 91/656 which was intended as a bioassay standard, was evaluated in this kit. Each ampule contains a nominal 1 µg of recombinant mouse IL-4, and was assigned an arbitrary unitage of 10,000 U/ampule.

NIBSC/WHO 91/656: 1 Unit of standard = 76 pg of Quantikine® Mouse IL-4.

SAMPLE VALUES

Serum - Forty individual mouse serum samples were evaluated for detectable levels of mouse IL-4 in this assay. All samples measured less than the lowest mouse IL-4 standard, 7.8 pg/mL.

Cell Culture Supernates:
Mouse splenocytes (2 x 10^6 cells/mL) were cultured for 3 days in RPMI supplemented with 10% fetal bovine serum and stimulated with lipopolysaccharide. An aliquot of the culture supernate was removed, assayed for mouse IL-4, and measured 42 pg/mL.

D10.G4.1 mouse helper T cells (1 x 10^5 cells/mL) were cultured for 4 days in RPMI supplemented with 10% fetal bovine serum, 50 µM β-ME, recombinant human IL-2 (10 ng/mL), irradiated C3H spleen cells (3 x 10^5 cells/mL) and conalbumin (100 µg/mL). An aliquot of the culture supernate was removed, assayed for mouse IL-4, and measured 2 ng/mL.

EL-4 mouse lymphoblast cells (9 x 10^5 cells/mL) were cultured for 2 days in DMEM supplemented with 10% fetal bovine serum and stimulated with 10 µg/mL PHA and 10 ng/mL PMA. An aliquot of the culture supernate was removed, assayed for mouse IL-4, and measured 23 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse IL-4.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range mouse IL-4 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

C10
G-CSF
GM-CSF
IFN-γ
IL-1α
IL-1β
IL-2
IL-3
IL-5
IL-6
IL-7
IL-9
IL-10
IL-10 R
IL-13
JE
KC
LIF
M-CSF
MIP-1α
MIP-1β
MIP-2
SCF
TNF-α
Tpo
VEGF

Recombinant rat:

IL-4

Recombinant human:

IL-4
IL-4 Rα

Recombinant mouse IL-4 R interferes at concentrations > 5 ng/mL.

REFERENCES

1. Benczik, M. and S.L. Gaffen (2004) Immunol. Invest. **33**:109.
2. Chomarat, P. and J. Banchereau (1998) Int. Rev. Immunol. **17**:1.
3. Lee, F. *et al.* (1986) Proc. Natl. Acad. Sci. USA **83**:2061.
4. Noma, Y. *et al.* (1986) Nature **319**:640.
5. Redfield, C. *et al.* (1991) Biochemistry **30**:11029.
6. Tatsenko, O.P. *et al.* (2004) Cytokine **28**:190.
7. Ramirez, F. *et al.* (1988) J. Immunol. Meth. **221**:141.
8. Leitenberg, D. and T.L. Feldbush (1988) Cell. Immunol. **111**:451.
9. Mosman, T.R. *et al.* (1987) J. Immunol. **138**:1813.
10. Chen, Y-T. and J.T. Kung (2007) J. Biomed. Sci. **14**:533.
11. Perona-Wright, G. *et al.* (2010) Nat. Immunol. **11**:520.
12. Gilmour, J. and P. Lavender (2008) Immunology **124**:437.
13. Paludan, S.R. (1998) Scand. J. Immunol. **48**:459.
14. Corthay, A. (2006) Scand. J. Immunol. **64**:93.
15. Ryan, J.J. *et al.* (2007) Crit. Rev. Immunol. **27**:15.
16. Grone, A. (2002) Vet. Immunol. Immunopathol. **88**:1.
17. Zhiguang, L. *et al.* (2009) Cell. Mol. Immunol. **6**:415.
18. Gocheva, V. *et al.* (2010) Genes Dev. **24**:241.