

Quantikine® ELISA

Human DPPIV/CD26 Immunoassay

Catalog Number DC260

For the quantitative determination of human Dipeptidyl Peptidase IV (DPPIV) concentrations in cell culture supernates, serum, plasma, saliva, and urine.

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

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INTRODUCTION

Dipeptidyl Peptidase IV (DPPIV; also known as CD26 and adenosine deaminase (ADA) complexing protein 2) is a serine protease that releases Xaa-Pro dipeptides from the N-terminus of oligo- and polypeptides (1). It is a type II membrane protein consisting of a small cytoplasmic tail, a transmembrane region, and a large extracellular domain. The extracellular domain contains glycosylation sites, a cysteine-rich region, and the catalytic active site (Ser, Asp and His charge relay system). In the native state, DPPIV is present as a non-covalently linked homodimer on the surface of a variety of cell types. The soluble form is also present in human serum and other body fluids. The form purified from human serum or seminal fluid corresponds to the intact extracellular domain (2).

DPPIV plays an important role in many physiological and pathological processes. It interacts with ADA and CD45, providing a co-stimulating signal to the CD3/T-cell receptor complex (3). It cleaves many chemokines with Xaa-Pro at their N-terminus, altering their receptor specificity and biological function (3). It degrades many peptide hormones, such as glucagon-like peptide-1, shorting their bioactivity. DPPIV inhibitors are being developed to extend their bioactivity and currently being tested in late-stage clinical trials for the treatment of type 2 diabetes (4). DPPIV truncates the N-terminus of procalcitonin, a marker for systemic bacterial and fungal infections (5). DPPIV interacts with HIV-1 Tat protein and its binding to ADA is inhibited by HIV envelop protein gp120 (6).

The Quantikine Human DPPIV/CD26 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human DPPIV in cell culture supernates, serum, plasma, saliva, and urine. It contains NS0-expressed recombinant human DPPIV, and antibodies raised against the recombinant factor. Results obtained using natural human DPPIV showed dose-response 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 naturally occurring human DPPIV.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human DPPIV has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any DPPIV present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human DPPIV is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of DPPIV bound in the initial step. The color development is stopped and the intensity of the color is measured.

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 the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

| PART | PART # | DESCRIPTION | STORAGE OF OPENED/ RECONSTITUTED MATERIAL |
|---------------------------|--------|--|--|
| Human DPPIV Microplate | 892951 | 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human DPPIV. | 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.* |
| Human DPPIV Conjugate | 892952 | 21 mL of polyclonal antibody specific for human DPPIV conjugated to horseradish peroxidase with preservatives. | May be stored for up to 1 month at 2-8 °C.* |
| Human DPPIV Standard | 892953 | 200 ng of recombinant human DPPIV in a buffer with preservatives; lyophilized. | |
| Assay Diluent RD1-57 | 895207 | 11 mL of a buffered protein base with blue dye and preservatives. | |
| Calibrator Diluent RD5K | 895119 | 21 mL of a animal serum with preservatives. <i>For cell culture supernate, saliva, and urine samples.</i> | |
| Calibrator Diluent RD5-33 | 895813 | 3 vials (21 mL/vial) of a buffered protein base with preservatives. <i>For serum/plasma samples.</i> | |
| Wash Buffer Concentrate | 895003 | 21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. <i>May turn yellow over time.</i> | |
| Color Reagent A | 895000 | 12 mL of stabilized hydrogen peroxide. | |
| Color Reagent B | 895001 | 12 mL of stabilized chromogen (tetramethylbenzidine). | |
| Stop Solution | 895032 | 6 mL of 2 N sulfuric acid. | |
| Plate Sealers | N/A | Adhesive strips. | |

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

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.
- Collection device for saliva samples that has no protein binding or filtering capabilities such as a Salivette® or equivalent.
- Test tubes for dilution of standards.
- Human DPPIV Controls (optional; available from R&D Systems).

PRECAUTIONS

DPPIV is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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

Some components in this kit contain ProClin® 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. Please 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 $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

Saliva - Collect saliva using a collection device such as a Salivette or equivalent. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Note: *Saliva collector must not have any protein binding or filtering capabilities.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum/plasma samples require a 100-fold dilution. A suggested 100-fold dilution is 10 μL of sample + 990 μL of Calbrator Diluent RD5-33.

Saliva samples require a 4-fold dilution. A suggested 4-fold dilution is 100 μL of sample + 300 μL of Calbrator Diluent RD5K.

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REAGENT PREPARATION

Bring all reagents to room temperature before use.

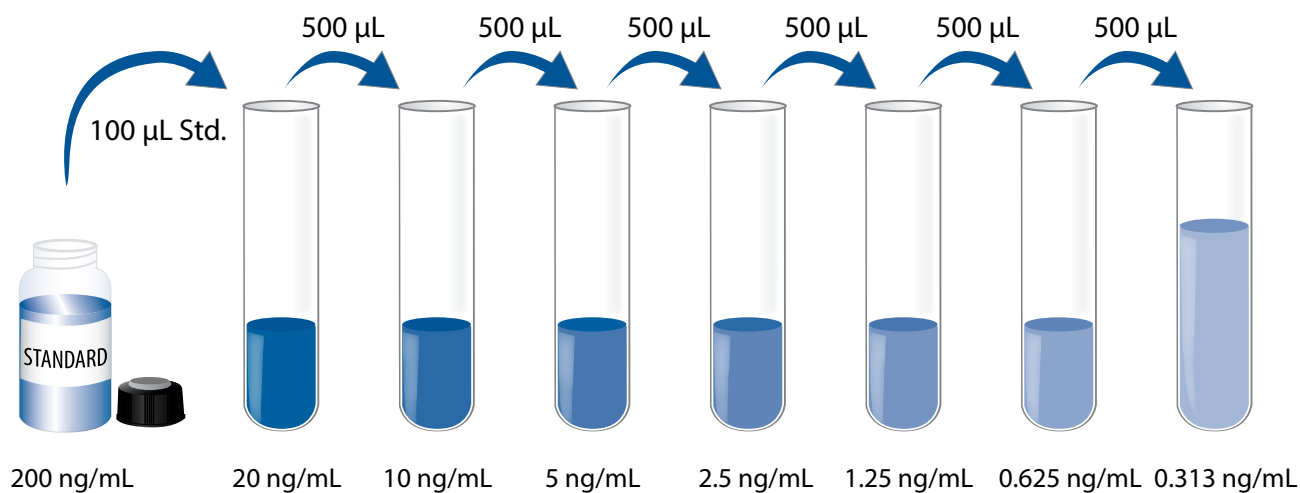
Note: *High concentrations of DPPIV are found in saliva. Use of a face mask and gloves to protect kit reagents from contamination is recommended.*

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 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. 200 μ L of the resultant mixture is required per well.

Human DPPIV Standard - Reconstitute the Human DPPIV Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 200 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 μ L of Calibrator Diluent RD5K (*for cell culture supernate, saliva and urine samples*) or Calibrator Diluent RD5-33 (*for serum/plasma samples*) into the 20 ng/mL tube. Pipette 500 μ L of the appropriate Calibrator Diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 20 ng/mL standard serves as the high standard. The appropriate Calibrator Diluent serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

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

Note: *High concentrations of DPPIV are found in saliva. Use of a face mask and gloves to protect kit reagents from contamination is recommended.*

1. Prepare all reagents, working standards, 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 100 μ L of Assay Diluent RD1-57 to each well.
4. Add 50 μ L of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 μ L) 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 200 μ L of Human DPPIV Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the benchtop.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 μ L of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 μ L of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, 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.).

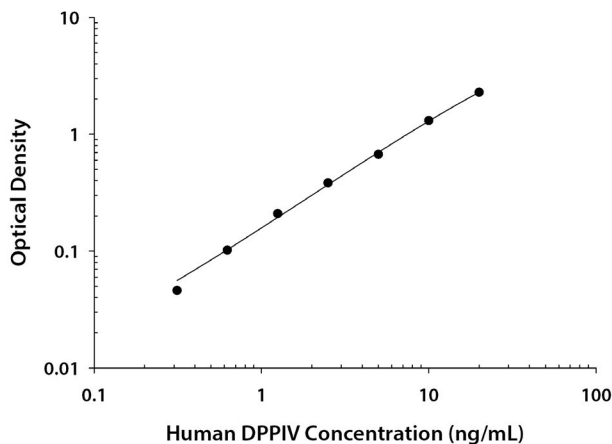
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human DPPIV 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

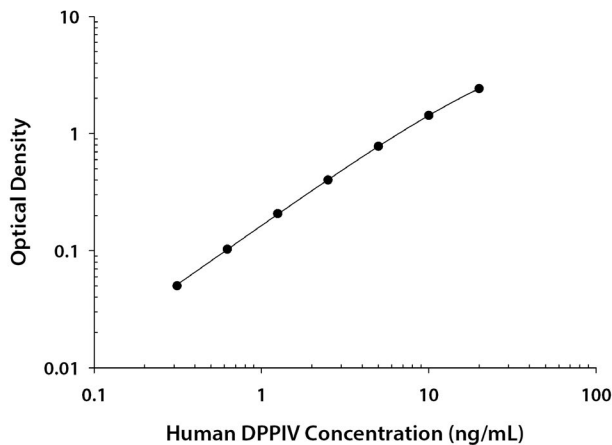
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE/SALIVA/URINE ASSAY



| (ng/mL) | O.D. | Average | Corrected |
|---------|----------------|---------|-----------|
| 0 | 0.010 0.010 | 0.010 | — |
| 0.313 | 0.055 0.056 | 0.056 | 0.046 |
| 0.625 | 0.110 0.114 | 0.112 | 0.102 |
| 1.25 | 0.214 0.223 | 0.219 | 0.209 |
| 2.5 | 0.385 0.401 | 0.393 | 0.383 |
| 5 | 0.656 0.707 | 0.682 | 0.672 |
| 10 | 1.307 1.330 | 1.319 | 1.309 |
| 20 | 2.284 2.297 | 2.291 | 2.281 |

SERUM/PLASMA ASSAY



| (ng/mL) | O.D. | Average | Corrected |
|---------|----------------|---------|-----------|
| 0 | 0.010 0.010 | 0.010 | — |
| 0.313 | 0.057 0.062 | 0.060 | 0.050 |
| 0.625 | 0.111 0.115 | 0.113 | 0.103 |
| 1.25 | 0.211 0.222 | 0.217 | 0.207 |
| 2.5 | 0.407 0.416 | 0.412 | 0.402 |
| 5 | 0.762 0.812 | 0.787 | 0.777 |
| 10 | 1.428 1.445 | 1.437 | 1.427 |
| 20 | 2.432 2.436 | 2.434 | 2.424 |

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 forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

CELL CULTURE SUPERNATE/SALIVA/URINE ASSAY

| | Intra-Assay Precision | | | Inter-Assay Precision | | |
|--------------------|-----------------------|-------|-------|-----------------------|-------|------|
| Sample | 1 | 2 | 3 | 1 | 2 | 3 |
| n | 20 | 20 | 20 | 40 | 40 | 40 |
| Mean (ng/mL) | 2.16 | 8.15 | 14.1 | 2.22 | 8.25 | 14.6 |
| Standard deviation | 0.105 | 0.280 | 0.916 | 0.140 | 0.695 | 1.18 |
| CV (%) | 4.9 | 3.4 | 6.5 | 6.3 | 8.4 | 8.1 |

SERUM/PLASMA ASSAY

| | Intra-Assay Precision | | | Inter-Assay Precision | | |
|--------------------|-----------------------|-------|------|-----------------------|-------|------|
| Sample | 1 | 2 | 3 | 1 | 2 | 3 |
| n | 20 | 20 | 20 | 40 | 40 | 40 |
| Mean (ng/mL) | 2.32 | 8.78 | 15.8 | 2.43 | 8.53 | 15.9 |
| Standard deviation | 0.142 | 0.354 | 1.15 | 0.207 | 0.777 | 1.30 |
| CV (%) | 6.1 | 4.0 | 7.3 | 8.5 | 9.1 | 8.2 |

RECOVERY

The recovery of human DPPIV spiked to levels throughout the range of the assay in various matrices was evaluated.

| Sample Type | Average % Recovery | Range |
|--------------------------|--------------------|-----------|
| Cell culture media (n=4) | 101 | 87 - 113% |
| Urine (n=4) | 101 | 88 - 113% |

LINEARITY

To assess linearity of the assay, samples containing and/or spiked with high concentrations of human DPPIV were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

| | | Cell culture supernates (n=4) | Serum* (n=4) | EDTA plasma* (n=4) | Heparin plasma* (n=4) | Urine (n=3) | Saliva (n=4) |
|------|-----------------------|-------------------------------|--------------|--------------------|-----------------------|-------------|--------------|
| 1:2 | Average % of Expected | 98 | 104 | 105 | 105 | 101 | 101 |
| | Range (%) | 87-110 | 101-107 | 99-108 | 102-108 | 99-102 | 99-102 |
| 1:4 | Average % of Expected | 96 | 107 | 105 | 105 | 101 | 103 |
| | Range (%) | 84-115 | 104-112 | 101-107 | 100-108 | 101-102 | 100-105 |
| 1:8 | Average % of Expected | 93 | 105 | 105 | 103 | 100 | 101 |
| | Range (%) | 83-112 | 100-110 | 101-110 | 98-108 | 97-103 | 94-107 |
| 1:16 | Average % of Expected | 95 | 103 | 100 | 98 | — | 95 |
| | Range (%) | 86-102 | 97-108 | 95-105 | 97-99 | — | 91-99 |

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

SENSITIVITY

Ninety-nine assays were evaluated and the minimum detectable dose (MDD) of human DPPIV ranged from 0.006-0.072 ng/mL. The mean MDD was 0.016 ng/mL.

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

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human DPPIV produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma/Urine/Saliva - Samples from apparently healthy volunteers were evaluated for the presence of human DPPIV in this assay. No medical histories were available for the donors used in this study.

| Sample Type | Mean (ng/mL) | Range (ng/mL) | Standard Deviation (ng/mL) |
|-----------------------|--------------|---------------|----------------------------|
| Serum (n=36) | 415 | 197 - 615 | 96.0 |
| EDTA plasma (n=36) | 391 | 187 - 604 | 85.4 |
| Heparin plasma (n=36) | 406 | 159 - 588 | 91.8 |
| Urine (n=10) | 6.06 | 2.26 - 13.3 | 3.35 |
| Saliva (n=10) | 32.6 | 13.0 - 69.9 | 17.9 |

Cell Culture Supernates:

Human peripheral blood cells (1×10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal calf serum, 50 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 μ g/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of natural human DPPIV.

| Condition | Day 1 (ng/mL) | Day 6 (ng/mL) |
|--------------|---------------|---------------|
| Unstimulated | ND | ND |
| Stimulated | ND | 0.697 |

ND=Non-detectable

HepG2 human hepatocellular carcinoma cells were cultured in MEM supplemented with 5% fetal calf serum until confluent and stimulated with 50 ng/mL PMA for 24 hours. An aliquot of the cell culture supernate was removed, assayed for natural human DPPIV, and measured 10.6 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human DPPIV.

The factors listed below were prepared at 200 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 20 ng/mL in a mid-range recombinant human DPPIV control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ACE
ACE-2
DPP-6
ECE-1
ECE-2
Neprilysin

Recombinant mouse:

CD26

REFERENCES

1. Misumi, Y. and Y. Ikehara (2004) in Handbook of Proteolytic Enzymes, Barrett, A.J. *et al.*, eds., Academic Press, San Diego, pp. 1905-1909.
2. Durinx, C. *et al.* (2000) Eur. J. Biochem. **267**:5608.
3. Sato, K. and N.H. Dang (2003) Int. J. Oncol. **22**:481.
4. Nielsen, L.L. (2005) DDT **10**:703.
5. Wrenger, S. *et al.* (2000) FEBS Lett. **466**:155.
6. Weihofen, W.A. *et al.* (2004) J. Biol. Chem. **279**:43330.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

| | | | | | | | | |
|----|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| 12 | | | | | | | | |
| 11 | | | | | | | | |
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| 3 | | | | | | | | |
| 2 | | | | | | | | |
| 1 | | | | | | | | |
| | A | B | C | D | E | F | G | H |

