FACULTAD DE MEDICINA
UNIVERSIDAD DE CHILE

Subdirección de Servicios

U. DE CHILE FAC. DE MEDICINA
OFICINA CENTRAL DE PARTES
27 MAR 2013
DOC: 39098

UNIVERSIDAD DE CHILE - CONTRALORÍA
INGRESO
19 MAR 2013

AUTORIZA TRATO DIRECTO CON CUSABIO.

UNIVERSIDAD DE CHILE
LEGALIDAD CONTROLADA
22 MAR 2013
CONTRALORIA

RESOLUCIÓN EXENTA N° 577

SANTIAGO, 18 MAR. 2013

VISTOS: Lo dispuesto en la Ley N° 19.886, sobre Bases de Contratos de Suministro y Prestación de Servicios; el Decreto Supremo N° 250 de 2004, que aprueba el Reglamento de la ley N° 19.886; el Estatuto de la Universidad de Chile contenido en el D.F.L. N° 153 de 1981, cuyo texto refundido, coordinado y sistematizado está contenido en el D.F.L. N°3 de 2006; en los Decretos Universitarios N° 2563 de 2010, N° 906 de 2009 y N° 2750 de 1980; y la Resolución N° 1600 de 2008 de la Contraloría General de la República;

CONSIDERANDO:

1. Que, el Programa de Genética, perteneciente a la Facultad de Medicina de la Universidad de Chile, requiere la adquisición de 1 Unidad Insulina Humana Ins Elisa Kit Modelo Csb-e05069h y 1 Unidad Glucagon Humano Gc Elisa Kit Modelo Csb-e9207h.

2. Que, lo anterior se fundamenta en lo señalado por el Director del Proyecto Anillo 1121 Dr. Pablo Caviedes, según carta de fecha 31 de Enero del 2013.

3. Que, es financiado por el centro ejecutante N°560731 – Pr. Anillo Act1121 Dr. Caviedes.

4. Que, el producto es distribuido por Cusabio.

5. Que, según lo indicado se configura la causal establecida en el N° 7 letra K del artículo 10 del Reglamento de la ley de compras; esto es "Cuando se trate de la compra de bienes y/o contratación de servicios que se encuentren destinados a la ejecución de proyectos específicos o singulares, de docencia, investigación o extensión, en que la utilización del procedimiento de licitación pública pueda poner en riesgo el objeto y la eficacia del proyecto de que se trata. En estos casos, las entidades determinarán por medio de una resolución, disponible en el Sistema de Información, los procedimientos internos que permitan resguardar la eficiencia, transparencia, publicidad, igualdad y no discriminación arbitraria en esta clase de adquisiciones", y la indicada en el Art. 3 N° 9 de la resolución 300 de 2010, de la Universidad de Chile.

6. Que, se adjunta documentación pertinente que sustenta el servicio (copia carta trato directo, cotización y solicitud N°345143).

7. Que, de acuerdo a lo anterior, dicto lo siguiente:

DIRECTOR JURÍDICO

RESOLUCIÓN:

1° AUTORÍZASE el pago a Cusabio, por la adquisición de 1 Unidad Insulina Humana Ins Elisa Kit Modelo Csb-e05069h y 1 Unidad Glucagon Humano Gc Elisa Kit Modelo Csb-e9207h, según cotización por un monto de US$ 1.385 dólares.

2° EMÍTASE la correspondiente orden de compra.

3° IMPÚTESE el gasto derivado de la presente Resolución al Título A Subtítulo 2 Ítem 2,6 del Presupuesto Universitario.

4° PUBLÍQUESE la presente Resolución en el Sistema de Información portal www.mercadopublico.cl.

5° ANÓTESE, COMUNÍQUESE Y ENVÍESE a Contraloría Universitaria para el respectivo control de legalidad.

DR. ENNIO VIVALDI VEJAR
VICEDECANO

DRA. CECILIA SEPÚLVEDA CARVAJAL
D E C A N A

DISTRIBUCIÓN:
- Subdirección de Servicios.
- OCEPA.
- CSC/EVV/BRG/jbp

Encargada:
Selene Jil Ponce
9786953

FACULTAD DE MEDICINA
UNIVERSIDAD DE CHILE

N° Solicitud	345143
SOLICITANTE	CAROLINAJARA
FECHA CREACIÓN	2013-01-31
EJECUTIVO	CAROLINA JARA
ENCARGADO ACTUAL	ABOGADO

BITACORA

Fecha	Hora	Usuario	Acción	Valor
2013-03-08	09:31	secretaria.servicios	Cambio de fase	Resolución devuelta para corrección
2013-03-07	16:17	direccion.juridica	Ingreso a despacho	Carpeta: D.Juridica => Subdirección de Servicios Jornada: Tarde
2013-03-06	18:02	direccion.juridica	Derivada	Encargado:abogado
2013-03-06	18:02	direccion.juridica	Cambio de fase	Enviada a Dirección Juridica
2013-03-06	15:06	secretaria.servicios	Derivada	Encargado:direccion.juridica
2013-03-06	15:06	secretaria.servicios	Cambio de fase	En evaluación Jurídica
2013-03-06	15:06	secretaria.servicios	Ingreso a despacho	Carpeta: Dirección Jurídica Jornada: Tarde
2013-01-31	21:24	admin.contratos	Derivada	Encargado:secretaria.servicios
2013-01-31	21:24	admin.contratos	Cambio de fase	Para confección - Resolución Trato Directo
2013-01-31	12:02	carolinajara	Derivada	Encargado:admin.contratos
2013-01-31	12:02	carolinajara	Cambio de fase	Para Preparación de Trato Directo
2013-01-31	11:07		carolinajara	Solicitud creada en modo borrador.
2013-01-31	11:08		carolinajara	Solicitud borrador actualizada.
2013-01-31	11:17	carolinajara		Solicitud Enviada. En espera de aprobacií¿½n.
2013-01-31	11:57	pcaviede	Validación de saldo	C.E:560731 Saldo a la fecha $ 137.440.093
2013-01-31	11:57	pcaviede	Solicitud aprobada	Por PABLO CAVIEDES con fecha: 2013-01-31 11:57:18
2013-01-31	11:57	pcaviede	Solicitud en proceso.	fecha esperada de término: 2013-01-31
2013-01-31	11:57	pcaviede	Cambio de fase	Enviada a Ejecutivo

OBSERVACIONES

Fecha	Hora	Usuario	Observación
2013-03-11	11:03	carolinajara	SE MODIFICA VALOR DE TRATO DIRECTO POR TRANSFERENCIA POR TOTAL DE US $1430, DADO QUE NO SE HABIA INCLUIDO GASTOS DE MANIPULACION Y MANTENCION COBRADOS POR EL BANCO DE CHINA.SE MODIFICA CARTA.

REFERENCIAS

No hay referencias.

COMPRA OTROS PRODUCTOS Y SERVICIOS (CHILECOMPRA)

CENTRO EJECUTANTE 560731 - PR. ANILLO ACT1121 DR. CAVIEDES

CENTRO REGISTRO 121312119102030 CENTRO COSTO 6812

TIPO DE COMPRA

TIPO DE COMPRA	COMPRAS DE 3 A 100 U.T.M
UTM A LA FECHA DE CREACIÓN	$ 40.005
CANTIDAD	2
MONTO APROXIMADO	$ 1
ENCARGADO DE SALA	
JUSTIFICACION Y/O OBJETIVO DE LA COMPRA	PARA REALIZAR INVESTIGACION EN EL PROYECTO ANILLO ACT1121

CRITERIOS DE EVALUACION

N°	CRITERIO	PORCENTAJE
1	PRECIO	40
2	CALIDAD TÉCNICA DE LOS BIENES O SERVICIOS	60
TOTAL		**100**

PROVEEDORES

NO HAY PROVEEDORES REGISTRADOS

CONTACTO TECNICO

CONTACTO TÉCNICO	CAROLINA JARA INOSTROZA
TELEFONO	9786946
EMAIL	CAROLINAJARA@MED.UCHILE.CL

DESPACHO

DIRECCION	INDEPENDENCIA #1027, PROGRAMA DE GENETICA,SECTOR C, 3ER. PISO

FACULTAD DE MEDICINA
UNIVERSIDAD DE CHILE

UNIVERSIDAD DE CHILE

Santiago, Enero 31 de 2013

Señora
Selene Jil Ponce
Encargada de Contratos
Faculta de Medicina
Universidad de Chile
Presente

Estimada Sra. Jil:

En cumplimiento a la normativa vigente de la Ley 19.886 de Compras Públicas, solicito a usted, tramite "**Resolución de Trato Directo**", con el proveedor CUSABIO (EN CHINA), por la suma de US$ 1430.-, correspondiente a la Compra de CSB-E05069h insulina humana INS ELISA kit y CSB-E09207h glucagon humano GC ELISA kit, (monto total incluye gastos por envío, US$ 100 cada kit por carga de China a Chile). Además el gasto total incluye cobro de manipulación y mantención que realiza Banco en China por US$ 45 (se adjunta correo electrónico con especificación de cobro). Solicitud Portal N° 345143. Se adjunta cotización S/N a través de correo electrónico enviado por Proveedor. El gasto es a cargo de mi Proyecto Anillo ACT1121, Ce. 560731

La transferencia se debe realizar a la siguiente cuenta:

Beneficiary: šWuhan huamei biotech Co.ï¼ŒLtd.
Address: šincubator building 4th floorï, ŒWuhan university Science Park. ŒNo.te-1 Daxueyuan Road, ŒDonghu Hi-tech development area, ŒWuhan ŒHubei province 430223, ŒP.R.China
Account No.: š200769730510029
Bank: Wuhan rural commercial bank
SWIFT code: WRCCCNBN
Address:Wuyin bldg room 906 No.618Jianshe avenue Wuhan China

Sin otro particular, saluda atentamente

Dr. Pablo Caviedes
Director Proyecto Anillo ACT1121
Programa de Farmacologia.

traducción do correo en ingles

Hola Pablo

Mi nombre es Joy Yan, representante de ventas de Cusabio, ahora responsable de los negocios en Estados Unidos.
Lamento que Yvonne dejó Cusabio durante mucho tiempo y espero que mi asistente podría ser de ayuda para usted!

Aquí debajo está la cotización FOB después de un 25% de descuento:
CSB-E05069h Insulina humana, INS ELISA Kit $ 592,5
CSB-E09207h glucagón humano, GC ELISA Kit $ 592,5

Envío de carga de China a Chile: $ 100 cada kit.
Cable de Transferencia de Información:

Beneficiario: šWuhan Huamei biotecnología Co.ï ¼ ŒLtd.
Dirección: Edificio šincubator cuarto floori, ŒWuhan universidad parque científico. ŒNo.te-1 Daxueyuan Road, ŒDonghu de alta tecnología zona de desarrollo, ŒWuhan ŒHubei provincia 430223, ŒP.R.China
Número de cuenta: š200769730510029
Banco: Wuhan rural banco comercial
Código SWIFT: WRCCCNBN
Dirección: Wuyin edif habitación 906 No.618Jianshe avenida Wuhan China

Además, aquí se adjunta es el manual para su referencia.
Cualquier pregunta, no dude en ponerse en contacto conmigo!

Mis mejores deseos!

Carolina Jara

De: Pablo Caviedes [pablo.caviedes@cicef.cl]
Enviado el: jueves, 31 de enero de 2013 9:18
Para: Carolina Jara
Asunto: Fwd: Re: Fw: Fwd: Re: From Cusabio Biotech Co.,Ltd
Datos adjuntos: CSB-E09207h.pdf; CSB-E05069h.pdf

Carolina:
A ver si alcanzas a ingresar esta orden, a la compañía CUSABIO en Chine. Se trata de dos kits ELISA, para determinación de hormonas.

Traduciendo, el costo es US$592.5 cada uno, más US$100 de flete por cada uno. Total: US$1385

Pablo Caviedes

-------- Mensaje original --------
Asunto: Re: Fw: Fwd: Re: From Cusabio Biotech Co.,Ltd
Fecha: Thu, 31 Jan 2013 10:10:34 +0800
De:america.joy <america.joy@cusabio.com>
Responder-a::america.joy <america.joy@cusabio.com>
A:pcaviede <pcaviede@med.uchile.cl>

COTIZACION

Hi Pablo

My name is Joy Yan, sales representative of Cusabio, now responsible for business in America.
I'm sorry that Yvonne left Cusabio for a long time and hope my assistant could be of help to you!

Here below is the FOB quotation after 25% discount:
CSB-E05069h Human Insulin,INS ELISA Kit $592.5
CSB-E09207h Human Glucagon,GC ELISA Kit $592.5

Shipping charge from China to Chile: $100 each kit.
Wire Transfer Information:

Beneficiary: Wuhan huamei biotech Co.,Ltd.
Address: incubator building 4th floor, Wuhan university science park. No.te-1 Daxueyuan Road, Donghu Hi-tech development area, Wuhan Hubei province 430223, P.R.China
Account No.: 200769730510029
Bank: Wuhan rural commercial bank
SWIFT code: WRCCCNBN
Address:Wuyin bldg room 906 No.618Jianshe avenue Wuhan China

Also, here enclosed is the manuals for your reference.
Any questions, feel free to contact me!

Best wishes!

Joy Yan

Sales&Marketing Dept. CUSABIO BIOTECH

Tel:+86 27 87928272 Fax:+86 27 87196150
Web:www.cusabio.com Email:america.joy@cusabio.com

From: Pablo Caviedes
Date: 2013-01-30 19:30
To: cusabio
CC: admin
Subject: Fwd: Re: From Cusabio Biotech Co.,Ltd

Dear Yvonne:
Have you had a chance to look at this request? Please let me know if you need anythong else

Best regards
Pablo Caviedes

-------- Mensaje original --------

Asunto: Re: From Cusabio Biotech Co.,Ltd
Fecha: Wed, 23 Jan 2013 13:16:24 -0400
De: Pablo Caviedes <pcaviede@med.uchile.cl>
OrganizaciÃ³n:ICBM, Fac. Medicine, Univ. of Chile
A:Yvonne <cusabio.caoyu@gmail.com>
CC:caoyu@cusabio.com, cusabio_caoyu@hotmail.com

Dear Yvonne:
I am wrting to request a quote for the following ELISA kits, including shipping. Also, please inclde forms of payment.

Product Name: Human Insulin,INS ELISA Kit, Cat# CSB-E05069h
Product Name: Human Glucagon,GC ELISA Kit:. Cat# CSB-E09207h

Very best regards
Pablo Caviedes

El 19-08-2010 2:54 a.m., Yvonne escribiÃ³:
Dear Pablo,
Good time.
It's been a long time not hearing from you.How's everything going around you?
Have you do the payment transfer yet?Recently,do you have any interest in any other ELISA Kits or any of our other products?
Await your soon reply!
Best regards
--
Yvonne
Cusabio Biotech Co.,Ltd
Tel: 86-27-87582341
Fax: 86-27-87196150
Mail: cusabio.caoyu@gmail.com
caoyu@cusabio.com
cusabio_caoyu@hotmail.com
Skepe:yvonne201081
Website: http://www.cusabio.com

--
Dr. Pablo A. Caviedes, M.D., Ph.D.
Program of Molecular & Clinical Pharmacology,
ICBM, Faculty of Medicine, Univ. of Chile
Independencia 1027.
Clasificador NÂ° 7, Independencia
Santiago, Chile
Phones: (562) 2978-6559 (Voice)
(562) 2737-2783 (FAX)
Email: pcaviede@med.uchile.cl

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Dr. Pablo A. Caviedes, M.D., Ph.D.
Director,
Center for Clinical Research &
Pharmacological Studies (CICEF)
Faculty of Medicine, Univ. of Chile
Independencia 1027.
Casilla 7- Clasificador 7 - Independencia
Código Postal: 8389100
Santiago, Chile
Phones: (562) 2978-6559 (Voice)
(562) 2737-2783 (FAX)
Email: pablo.caviedes@cicef.cl

Carolina Jara

Asunto: RV: RV: Re: Fw: Fwd: Re: From Cusabio Biotech Co.,Ltd

Hola Carolina,

Gracias por tu recuerdo y sí, la transferencia total debe incluir los gastos de manipulación preguntó por el banco.
En términos generales, alrededor de $ 45 se le preguntó por el banco para cada transferencia.
Por lo tanto, la cantidad total de transferencia debería ser de $ 1385 + $ 45 = $ 1430.

Si tiene alguna pregunta, no dude en contactarnos!

Saludos cordiales,

De: america.joy [mailto:america.joy@cusabio.com]
Enviado el: viernes, 08 de marzo de 2013 20:44
Para: Carolina Jara
Asunto: Re: RV: Re: Fw: Fwd: Re: From Cusabio Biotech Co.,Ltd

Hi Carolina,

Thanks for your remind and yes, the total transfer should include handling charge asked by the bank.
Generally speaking, about $45 would be asked by the bank for each transfer.
So, the total transfer amount should be $1385+$45=$1430.

Should you have any questions, feel free to contact!

Best regards,

Joy Yan

From: Carolina Jara
Date: 2013-03-09 04:10
To: america.joy@cusabio.com
Subject: RV: Re: Fw: Fwd: Re: From Cusabio Biotech Co.,Ltd

Hi Joy:

second attachment, please indicate if you appreciate within amounts listed, charges that are covered by the Bank for living expenses. Please indicate appreciate information as sent second quote we should make a total transfer: U.S. $ 1,385. appreciate indicate whether that contribution should be added to any other charge that is not covered.

thanking information.

greets atte.

Carolina Jara Inostroza
Ejecutiva de Proyectos
Sub Dirección de Servicios
Facultad de Medicina - Universidad de Chile

carolinajara@med.uchile.cl
Fono 978 69 46
Fax 978 69 54.

De: Pablo Caviedes [mailto:pablo.caviedes@cicef.cl]
Enviado el: jueves, 31 de enero de 2013 9:18
Para: Carolina Jara
Asunto: Fwd: Re: Fw: Fwd: Re: From Cusabio Biotech Co.,Ltd

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A:pcaviede <pcaviede@med.uchile.cl>

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CSB-E09207h Human Glucagon,GC ELISA Kit $592.5

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Address: šincubator building 4th floorï, ŒWuhan university science park. ŒNo.te-1 Daxueyuan Road, ŒDonghu Hi-tech development area, ŒWuhan ŒHubei province 430223, ŒP.R.China
Account No.: š200769730510029
Bank: Wuhan rural commercial bank
SWIFT code: WRCCCNBN
Address:Wuyin bldg room 906 No.618Jianshe avenue Wuhan China

Also, here enclosed is the manuals for your reference.
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Best wishes!

Joy Yan

Sales&Marketing Dept. CUSABIO BIOTECH

Tel:+86 27 87928272 Fax:+86 27 87196150
Web:www.cusabio.com Email:america.joy@cusabio.com

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CC: admin
Subject: Fwd: Re: From Cusabio Biotech Co.,Ltd

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Best regards
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De: Pablo Caviedes <pcaviede@med.uchile.cl>
OrganizaciÃ³n:ICBM, Fac. Medicine, Univ. of Chile
A:Yvonne <cusabio.caoyu@gmail.com>
CC:caoyu@cusabio.com, cusabio_caoyu@hotmail.com

Dear Yvonne:
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Product Name: Human Insulin,INS ELISA Kit, Cat# CSB-E05069h
Product Name: Human Glucagon,GC ELISA Kit:. Cat# CSB-E09207h

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El 19-08-2010 2:54 a.m., Yvonne escribiÃ³:
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Await your soon reply!
Best regards
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Yvonne
Cusabio Biotech Co.,Ltd
Tel: 86-27-87582341
Fax: 86-27-87196150
Mail: cusabio.caoyu@gmail.com
caoyu@cusabio.com
cusabio_caoyu@hotmail.com
Skepe:yvonne201081
Website: http://www.cusabio.com

--
Dr. Pablo A. Caviedes, M.D., Ph.D.
Program of Molecular & Clinical Pharmacology,
ICBM, Faculty of Medicine, Univ. of Chile
Independencia 1027.
Clasificador NÂ° 7, Independencia
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Dr. Pablo A. Caviedes, M.D., Ph.D.
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Casilla 7- Clasificador 7 - Independencia
Código Postal: 8389100
Santiago, Chile
Phones: (562) 2978-6559 (Voice)
(562) 2737-2783 (FAX)
Email: pablo.caviedes@cicef.cl

Human glucagon (GC) ELISA Kit

Catalog Number. CSB-E09207h

For the quantitative determination of human glucagon (GC) concentrations in serum, plasma, tissue homogenates.

This package insert must be read in its entirety before using this product.

If You Have Problems

Technical Service Contact information

Phone: 86-27-87582341
Fax: 86-27-87196150
Email: tech@cusabio.com
Web: www.cusabio.com

In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for GC has been pre-coated onto a microplate. Standards and samples are pipetted into the wells with a Horseradish Peroxidase (HRP) conjugated antibody specific for GC. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of GC bound in the initial step. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

15 pg/ml-200 pg/ml.

SENSITIVITY

The minimum detectable dose of human GC is typically less than 5 pg/ml.
The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest human GC concentration that could be differentiated from zero. It was determined the mean O.D value of 20 replicates of the zero standard added by their three standard deviations.

SPECIFICITY

This assay has high sensitivity and excellent specificity for detection of human GC. No significant cross-reactivity or interference between human GC and analogues was observed.

Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between human GC and all the analogues, therefore, cross reaction may still exist.

PRECISION

Intra-assay Precision (Precision within an assay): CV%<15%
Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV%<15%
Three samples of known concentration were tested in twenty assays to assess.

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, dilute the samples and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Reagents	Quantity
Assay plate	1 (96 wells)
Standard	6 (lyophilized)
HRP-conjugate	1 x 6 ml
Wash Buffer (20 x concentrate)	1 x 15 ml
Substrate A	1 x 7 ml
Substrate B	1 x 7 ml
Stop Solution	1 x 7 ml
Adhesive Strip (For 96 wells)	4
Instruction manual	1

STANDARD CONCENTRATION

Standard	S0	S1	S2	S3	S4	S5
Concentration (pg/ml)	0	15	30	58	100	200

STORAGE

Unopened kit	Store at 2 - 8°C. Do not use the kit beyond the expiration date.
Opened kit	May be stored for up to 1 month at 2 - 8° C.

***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 600 nm - 630 nm.
- An incubator which can provide stable incubation conditions up to 37°C±0.5°C.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- 100 mL and 500 mL graduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

SAMPLE COLLECTION AND STORAGE

- **Serum** Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1000 ×g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
- **Plasma** Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 ×g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
- **Tissue Homogenates** 100mg tissue was rinsed with 1X PBS, homogenized in 1 ml of 1X PBS and stored overnight at -20°C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g, 2 - 8°C. The

supernate was assayed and removed immediately. Alternatively, aliquot and store samples at -20°C or -80°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

Note:

1. CUSABIO is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤1month) or -80°C (≤2month) to avoid loss of bioactivity and contamination.
3. Grossly hemolyzed samples are not suitable for use in this assay.
4. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
5. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
6. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
7. Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
8. Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
9. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

REAGENT PREPARATION

Note:

- **Kindly use graduated containers to prepare the reagent.**
- Bring all reagents to room temperature (18-25°C) before use for 30min.
- Distilled water is recommended to be used to make the preparation for reagents or samples. Contaminated water or container for reagent preparation will influence the detection result.

1、**Wash Buffer(1x)**- If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 15 ml of Wash Buffer Concentrate (20 x) into deionized or distilled water to prepare 300 ml of Wash Buffer (1 x).

2、**Standard**

Centrifuge the standard vial at 6000-10000rpm for 30s.
Reconstitute each lyophilized **Standard(S0-S5)** with 0.5 ml of **ddH_2O**. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to use.

ASSAY PROCEDURE

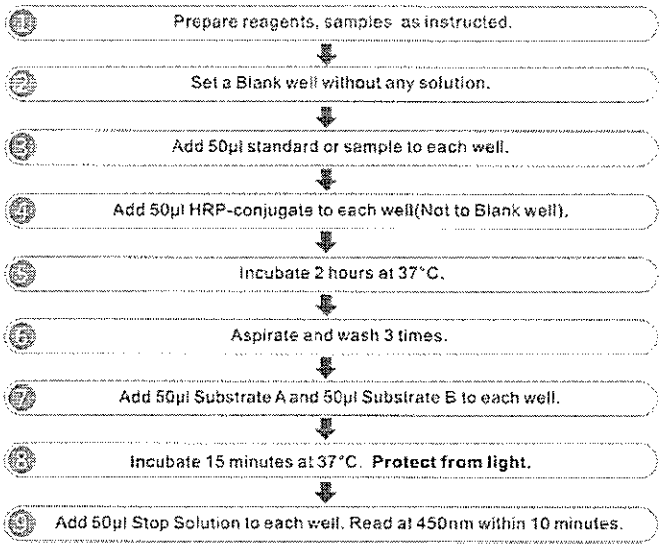
Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, standards and samples as directed in the previous sections.
2. Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Set a Blank well without any solution.
4. Add 50µl of **Standard** or **Sample** per well. Standard need test in duplicate.
5. Add 50µl of **HRP-conjugate** to each well (not to Blank well). Mix well and then incubate for 2 hours at 37°C.
6. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with **Wash Buffer** (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 10 seconds, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating ordecanting. Invert the plate and blot it against clean paper towels.
7. Add 50µl of **Substrate A** and 50µl of **Substrate B** to each well, mix well. Incubate for 15 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
8. Add 50µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. 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.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. 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.
5. Controlling of reaction time: Observe the change of color after adding Substrates (e.g. observation once every 10 minutes). Substrates should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. Substrates are easily contaminated. Substrates should remain colorless or light blue until added to the plate. Please protect it from light.
7. Stop Solution should be added to the plate in the same order as the Substrates. 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 Substrates.

ASSAY PROCEDURE SUMMARY



CALCULATION OF RESULTS

Using the professional soft "Curve Expert 1.3" to make a standard curve is recommended, which can be downloaded from our web.

Average the duplicate readings for each standard and sample and subtract the average optical density of Blank.

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 x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the GC 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.

Human insulin (INS) ELISA Kit

Catalog Number. CSB-E05069h

For the quantitative determination of human insulin (INS) concentrations in serum, plasma, cell culture supernates, tissue homogenates.

This package insert must be read in its entirety before using this product.

If You Have Problems

Technical Service Contact information

Phone: 86-27-87582341
Fax: 86-27-87196150
Email: tech@cusabio.com
Web: www.cusabio.com

In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for INS has been pre-coated onto a microplate. Standards and samples are pipetted into the wells with a Horseradish Peroxidase (HRP) conjugated antibody specific for INS. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of INS bound in the initial step. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

8 μIU/ml-140 μIU/ml.

SENSITIVITY

The minimum detectable dose of human INS is typically less than 5 μIU/ml.
The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest human INS concentration that could be differentiated from zero. It was determined the mean O.D value of 20 replicates of the zero standard added by their three standard deviations.

SPECIFICITY

This assay has high sensitivity and excellent specificity for detection of human INS. No significant cross-reactivity or interference between human INS and analogues was observed.

Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between human INS and all the analogues, therefore, cross reaction may still exist.

PRECISION

Intra-assay Precision (Precision within an assay): CV%<15%
Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV%<15%
Three samples of known concentration were tested in twenty assays to assess.

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, dilute the samples and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Reagents	Quantity
Assay plate	1(96 wells)
Standard	1 x 0.5 ml / 5(lyophilized)
HRP-conjugate	1 x 6 ml
Wash Buffer (20 x concentrate)	1 x 15 ml
Substrate A	1 x 7 ml
Substrate B	1 x 7 ml
Stop Solution	1 x 7 ml
Adhesive Strip (For 96 wells)	4
Instruction manual	1

STANDARD CONCENTRATION

Standard	S0	S1	S2	S3	S4	S5
Concentration (µIU/ml)	0	8	16	32	80	140

STORAGE

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

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 600 nm - 630 nm.
- An incubator which can provide stable incubation conditions up to 37°C±0.5°C.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- 100 mL and 500 mL graduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.

PRECAUTIONS

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SAMPLE COLLECTION AND STORAGE

- **Serum** Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1000 ×g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
- **Plasma** Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 ×g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernates** Remove particulates by centrifugation for 15 minutes at 1000 x g, 2 - 8°C and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
- **Tissue Homogenates** 100mg tissue was rinsed with 1X PBS, homogenized in 1 ml of 1X PBS and stored overnight at -20°C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g, 2 - 8°C. The supernate was assayed and removed immediately. Alternatively, aliquot and store samples at -20°C or -80°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

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