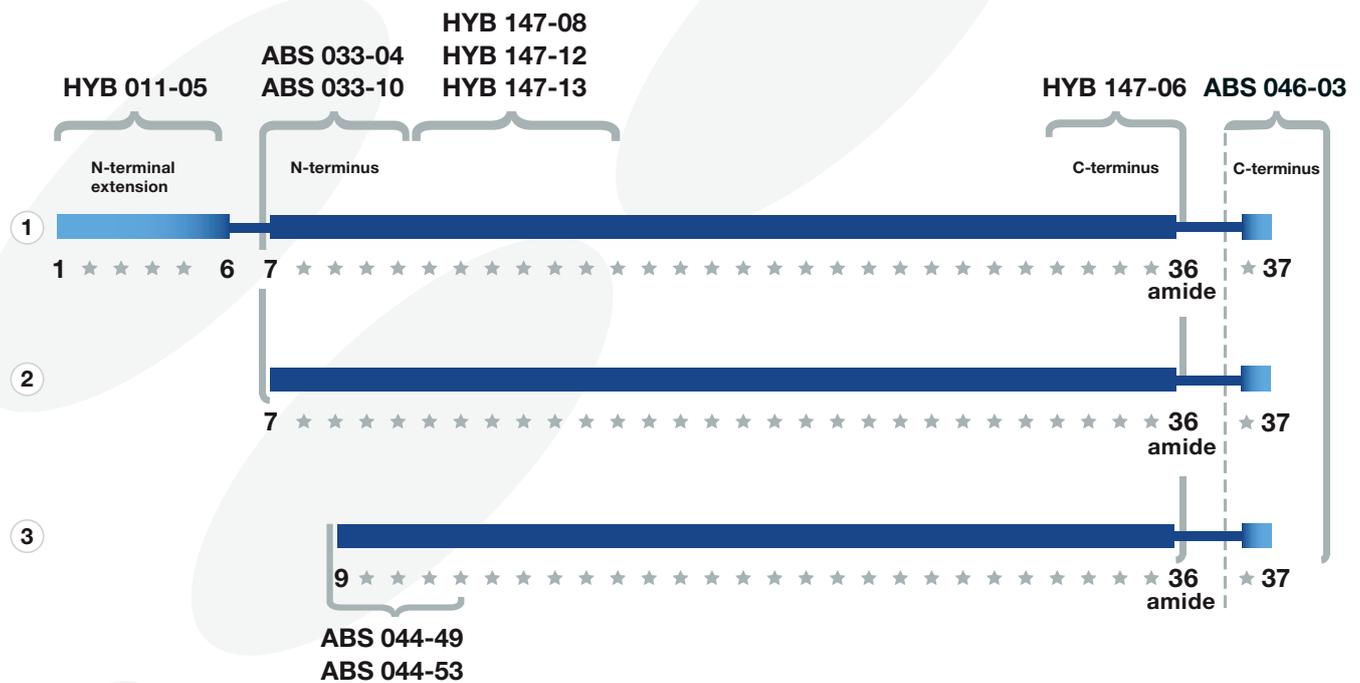


Research Antibodies for Diabetes and Obesity

GLP-1 IN FOCUS

Glucagon-like peptide-1



- ① GLP-1 Extended form
- ② GLP-1 Active form
- ③ GLP-1 Metabolite

GLP-1 MONOCLONAL ANTIBODIES

| Cat. No. | Specificity |
|-------------|--|
| HYB 011-05 | Glucagon-like peptide-1 (GLP-1(1-37) and GLP-1(1-36) amide, N-terminal-extension specific) |
| ABS 033-04 | Glucagon-like peptide-1 (GLP-1(7-37) and GLP-1(7-36)amide, N-terminal specific) |
| ABS 033-10* | Glucagon-like peptide-1 (GLP-1(7-37) and GLP-1(7-36)amide, N-terminal specific) |
| HYB 147-08 | Glucagon-like peptide-1 (GLP-1, Mid-molecule specific) |
| HYB 147-12* | Glucagon-like peptide-1 (GLP-1, Mid-molecule specific) |
| HYB 147-13 | Glucagon-like peptide-1 (GLP-1, Mid-molecule specific) |
| HYB 147-06* | Glucagon-like peptide-1 (GLP-1 (7-36)amide, C-terminal specific) |
| ABS 044-49 | Glucagon-like peptide-1 (GLP-1(9-37) and GLP-1(9-36)amide, N-terminal specific) |
| ABS 044-53 | Glucagon-like peptide-1 (GLP-1(9-37) and GLP-1(9-36)amide, N-terminal specific) |
| ABS 046-03* | Glucagon-like peptide-1 (GLP-1, Non-amidated (-Arg-Gly), C-terminal specific) |

* Also available biotinylated

GLP-1 in focus

Treating type-2 diabetes mellitus and obesity

GLP-1 (glucagon-like peptide-1) remains hot news in treating type-2 diabetes and obesity. Several long-acting GLP-1 receptors agonist have been developed, including exendin-4 and exendin-4 derivatives, and dipeptidyl peptidase-4 (DPP-4) inhibitors which act by reducing the breakdown of endogenous GLP-1, are coming into increasing use, adding further impetus to the interest in measuring the active forms of GLP-1. All these agents reduce the glycemic response to meals, an effect that is attributable to increased insulin secretion and inhibitory effects on the gastrointestinal tract. Gratifyingly, at a time when obesity is seen as an increasing health problem, some of these treatments have also produced a significant weight loss in experimental animals and human subjects.

Measuring the active forms of GLP-1

GLP-1 is the most potent endogenous stimulator of insulin release in response to food. Determining plasma levels is a problem, as its active forms, GLP-1(7-36)amide and GLP-1(7-37), need terminal-specific antibodies to distinguish them not only from larger, inactive precursor fragments (such as GLP-1(1-36)amide and MPPGF, major proglucagon fragment), but also from their inactive degradation products, GLP-1(9-36)amide and GLP-1(9-37). These are rapidly produced by the action of DPP-4, which is chiefly responsible for the very short half-life of the native active forms in the circulation (and subsequently in blood specimens for analysis). At the same time, circulating levels of the native active forms peak in the low picomolar range, requiring sensitive assay methods for their determination.

AntibodyShop monoclonal antibodies to GLP-1

AntibodyShop offers a range of monoclonal antibodies to GLP-1, directed against different epitopes in the active forms. Their characteristics and possible uses are commented on below.

Binding to the free N-terminus of GLP-1(7-36)amide and GLP-1(7-37)

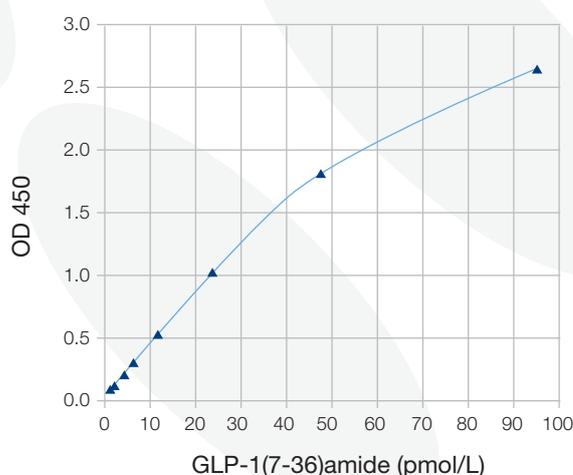
ABS 033-04 and ABS 033-10: These antibodies are highly specific to the free, unextended and untruncated N-terminus of the active forms of GLP-1. In inhibition ELISA they show 0.25% or less cross-reactivity with either the N-terminally extended GLP-1(1-36)amide or the inactive N-terminally truncated products of DPP-4 digestion. Whereas ABS 033-04 does not cross-react appreciably with GLP-1-related peptides, ABS 033-10 cross-reacts about 50% with exendin-4 and can in fact be used as a capture antibody for exendin-4 in sandwich ELISA.

While it could be hoped that these antibodies could be used as capture antibodies to measure both active forms of GLP-1 in a single sandwich ELISA, ABS 033-04 functions poorly as a capture antibody in unoptimized assay set-ups, giving assays that are too insensitive to measure physiological levels of the active peptides. The binding characteristics of this antibody appear to be substantially modified on adsorption to polystyrene.

Binding to the free N-terminus of GLP-1(9-36)amide and GLP-1(9-37)

ABS 044-49 and ABS 044-53 are two new antibodies that recognize the N-terminus of the GLP-1 degradation products by DPP-4, GLP-1(9-36)amide and GLP-1(9-37). ABS 044-49 cross-reacts 3% with GLP-1(7-36)amide while the cross reaction seen with ABS 044-53 is less than 2%.

FIGURE 1



Sandwich ELISA for C-terminally amidated forms of GLP-1

This uses HYB 147-06 as capture antibody in combination with biotinylated detection antibody HYB 147-12 to measure the sum of GLP-1(7-36)amide, GLP-1(1-36)amide and GLP-1(9-36)amide in biological samples. Relative to measuring the two active forms of GLP-1, the responses are reduced by not measuring the contribution of GLP-1(7-37) to the overall response, but this is compensated for by measuring an approximately equal contribution from GLP-1(1-36)amide. However, the GLP-1(9-36)amide degradation product is also measured, augmenting the response above that of the active forms alone. This assay cannot detect changes in active GLP-1 in response to treatment with DPP-4 inhibitors, and is therefore only suitable for experiments in which changes in GLP-1 degradation are irrelevant.

Binding to the mid-molecular region of GLP-1(7-36)amide and GLP-1(7-37)

HYB 147-08, HYB 147-12 and HYB 147-13: These antibodies bind to epitopes located within the region defined by residues 11-32 of GLP-1. They block each other's binding to a significant but slightly varying extent, suggesting that the epitopes are close enough for steric hindrance of antibody binding to come into play. It is to be expected that these "side-reading" GLP-1 antibodies also react with larger GLP-1-containing precursor fragments, such as major proglucagon fragment, which circulates in the blood at levels greatly in excess of the physiological levels of the active forms

of GLP-1. They also cross-react fully with the products of DPP-4 digestion, GLP-1(9-36)amide and GLP-1(9-37). This side-reading, mid-molecular binding characteristic means that their capture of the active peptides in sandwich ELISA is impaired by the presence of higher concentrations of inactive fragments. However, they are often excellent detection antibodies.

Binding to the free amidated C-terminus of GLP-1(7-36)amide and GLP-1(1-36)amide

HYB 147-06: This unique monoclonal antibody binds specifically to the amidated C-terminus of GLP-1 in both the active peptide GLP-1(7-36)amide and the corresponding N-terminally extended or truncated peptides, GLP-1(1-36)amide and GLP-1(9-36)amide. It performs very well as a capture antibody for these C-terminally amidated peptides and shows less than 0.1% cross-reactivity with the non-amidated forms (37Gly-OH).

Binding to the non-amidated (-Arg-Gly), C-terminal of GLP-1

ABS 046-03 binds GLP-1(1-37), GLP-1(7-37) and GLP-1(9-37). A sandwich setup consisting of ABS 046-03 as a capture antibody with ABS 033-10 as a biotinylated detection antibody can be used to measure non-amidated GLP-1. This setup cross-reacts about 5% with C-terminally amidated GLP-1.

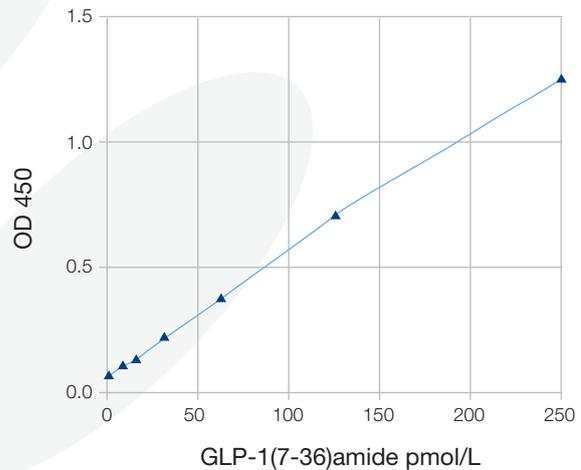
Sandwich ELISAs for physiologically relevant forms of GLP-1

Figures 1 and 2 show ELISAs for C-terminally amidated forms of GLP-1 and for the principal active form, GLP-1(7-36)amide.

Polyclonal/monoclonal sandwich ELISAs

In addition, antibodies HYB 147-06, HYB 147-12 and ABS 033-10 can be used as detection antibodies with a polyclonal antibody capture coat, to create sandwich assays that superimpose the specificity of the detection antibody on that of the capture antibody employed.

FIGURE 2



Sandwich ELISA specific for GLP-1(7-36) amide, the principal active form

This uses HYB 147-06 as capture antibody in combination with biotinylated detection antibody ABS 033-10 exclusively to measure GLP-1(7-36)amide in biological samples. GLP-1(7-36)amide is the principal active form of the peptide, and has been estimated to make up about 70% of the physiological response. Relative to measuring both active forms of GLP-1 together, the responses are reduced by not measuring the approximately 30% contribution of GLP-1(7-37) to the overall response. There is no cross-reactivity with any extended or truncated physiological form of GLP-1(7-36)amide. Specimens must be collected into tubes containing an adequate amount of DPP-4 inhibitor (in addition to routine peptidase inhibitors) to prevent the rapid in vitro degradation of the active peptide.

Exendin-4 in focus

Treating type-2 diabetes mellitus and obesity

Exendin-4, a GLP-1-like peptide from Gila monster venom, is a naturally occurring long-acting GLP-1 receptor agonist. As such, it has been one of the prime candidates in new treatments for type-2 diabetes and obesity, along with derivatives either of itself or of GLP-1. An exendin-4 product has in fact been in use for treating type-2 diabetes for some years. By stimulating glucose-dependent insulin secretion and exerting other effects on the gastrointestinal tract, exendin-4 and its analogues reduce the glycemic response to meals and may be associated with significant weight loss.

Measuring Exendin-4

As exendin-4 is not an endogenous peptide but a pharmacological agent in mammals, its measurement by immunochemical techniques has received less attention than measuring the active forms of the native peptide hormone GLP-1, on whose receptor it acts. However, the truncated exendin fragment 9-39, also used experimentally, is a GLP-1 receptor anta-agonist. It is convenient if immunochemical

techniques to measure exendin-4 do not measure related glucagon-like peptides, and in some cases it is desirable to distinguish intact exendin-4 from its N-terminally truncated fragments. There is a possibility that N-terminally reacting antibodies may cross-react with the N-terminus of GLP-1(7-36)amide, as the N-terminus is the site of greatest sequence similarity between the peptides.

AntibodyShop monoclonal antibodies to Exendin-4

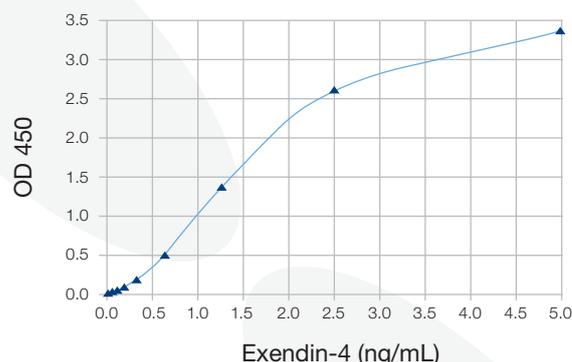
Two types of antibodies against exendin-4 are available. The ABS 012 series (-20, -23, -24 and -35) were raised against intact exendin-4 and all bind to an epitope in the 9-39 region, mutually inhibiting each other's binding. These antibodies do not cross-react with other glucagon-related peptides tested. The other antibody, ABS 033-10, was gene-rated against an N-terminal fragment of GLP-1, but shows a 50% cross-reaction with exendin-4, presumably reacting with the free N-terminal. This antibody reacts with the intact active forms of GLP-1.

EXENDIN-4 MONOCLONAL ANTIBODIES

| Cat. No. | Specificity |
|-------------|-------------|
| ABS 012-20 | Exendin-4 |
| ABS 012-24 | Exendin-4 |
| ABS 012-35* | Exendin-4 |

* Also available biotinylated

FIGURE 3



Sandwich ELISA for N-terminally intact Exendin-4

By using the above monoclonal antibodies, it is possible to measure intact exendin-4 by a sandwich ELISA which is not subject to interference from GLP-1 or other glucagon-related peptides that may be present. ABS 033-10 is used as capture antibody in combination with a biotinylated detection antibody from the ABS 012 series. The best sensitivity in an unoptimized buffer assay was obtained with biotinylated ABS 012-35 as the detection antibody.

RELATED PRODUCTS - MOUSE MONOCLONAL ANTIBODIES

| Cat. No. | Specificity |
|----------|---|
| ABS 020 | Peptide histidine-methionine (human, PHM) |
| ABS 021 | Gastric inhibitory polypeptide (human, hGIP) |
| ABS 022* | Leptin (human) |
| ABS 023 | Vasoactive intestinal peptide (VIP) |
| ABS 026 | α -CGRP (human, α -calcitonin gene-related peptide) |
| ABS 027 | Adrenomedullin (human) |
| ABS 028 | Neuropeptide Y (human, rat, NPY) |
| ABS 029* | Peptide YY (human, PYY) |

| Cat. No. | Specificity |
|--------------|---|
| ABS 051-04 | PYY (3-36)(peptide tyrosine-tyrosine amide, 3-36) |
| ABS 030 | Pancreatic Polypeptide (human, PP) |
| HYB 006 | Glucagon-like peptide-2 (human, hGLP-2) |
| ABS 052-121* | Ghrelin (human, rat) |
| ABS 050-45 | Ghrelin (human) |
| ABS 058-01* | Glucagon (human, pig, rat) |
| ABS 061-09 | Glucagon (human, pig, rat) |

* Also available biotinylated

For more information, please contact BioPorto Diagnostics or your local BioPorto Diagnostics distributor.



BioPorto Diagnostics A/S
 Grusbakken 8
 DK-2820 Gentofte
 Denmark

Phone (+45) 4529 0000
 Fax (+45) 4529 0001
 E-mail info@bioporto.com
 Web www.bioporto.com
 www.ngal.com

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Beratung und Vertrieb: dianova GmbH
 Tel: 040 - 45067 0 • Fax: 040 - 450 45067 490
 Warburgstr. 45 • 20354 Hamburg • www.dianova.de