GAD65 and IA-2 Antigens

Insulin-dependent diabetes mellitus (IDDM) or type 1 diabetes mellitus (T1DM) is a T-cell mediated autoimmune disorder characterized by destruction of pancreatic beta cells (Ziegler *et al.* 2013). Mostly starting in childhood, this leads to insulin deficiency and metabolic abnormalities (Pihoker *et al.* 2005). Patients require lifelong insulin treatment (Landin-Olsson *et al.* 1992).

In the 1970s it was described that beta cell destruction is associated with the production of autoantibodies to cytoplasmic antigens of islet cells (ICAs) (Bottazzo *et al.* 1974). Using classic diagnostic ICA tests, polyclonal antibodies are detected in approximately 85% of children with recently diagnosed T1DM (Winter *et al.* 2002).

In the early 1990s, new antigens were identified including a 37/40 kDa tryptic fragment, which was identified to belong to the putative tyrosine phosphatase insulinoma-associated protein (IA-2), as well as the glutamate decarboxylase (GAD) antigen (Bækkeskov *et al.* 1990; Passini *et al.* 1995).

GAD is a pyridoxal phosphate-dependent enzyme that catalyzes the irreversible decarboxylation of glutamate to form gamma-aminobutyrate (GABA). Named according to its respective molecular weight, the pancreatic GAD65 isoform contains an N-terminal membrane-anchoring signal peptide and localizes in the proximity of the Golgi apparatus of islet cells and GABA-containing vesicles (Brilliant *et al.* 1990; Bu *et al.* 1992; Solimena *et al.* 1994). GAD65 autoantibodies appear in 70–80% of sera from recently diagnosed T1DM patients (Hagopian *et al.* 1993). However, they can also be present in nondiabetic individuals and are thus alone not specific (Christie *et al.* 1994).

IA-2, also called islet cell antigen 512 (ICA 512), is a catalytically inactive protein tyrosine phosphatase (PTP) (Bonifacio *et al.* 1995). It consists of an N-terminal extracellular signal sequence, a transmembrane domain and a long C-terminal intracellular tail, that harbors the majority of autoantibody epitopes (Lampasona *et al.* 1996). Similar to GAD it is expressed within secretory granules in neural, neuroendocrine and pancreatic islet cells (Solimena *et al.* 1996). Compared to anti-GAD antibodies, anti-IA-2 antibodies appear later and are therefore used as predictive value for upcoming T1DM onset in at-risk individuals (Achenbach *et al.* 2013). IA-2 autoantibodies are detected in 60–80% of sera from individuals with recent onset of the disease (Winter *et al.* 2011).

DIARECT's antigens, GAD65 and IA-2 (ICA 512), are produced in the baculovirus/insect cell expression system.

Ordering Information		
31900	Glutamate Decarboxylase 65 kDa	0.1 mg
31901	(GAD65; ng)	1.0 mg
30500	IA-2 (ICA 512)	0.1 mg
30501		1.0 mg

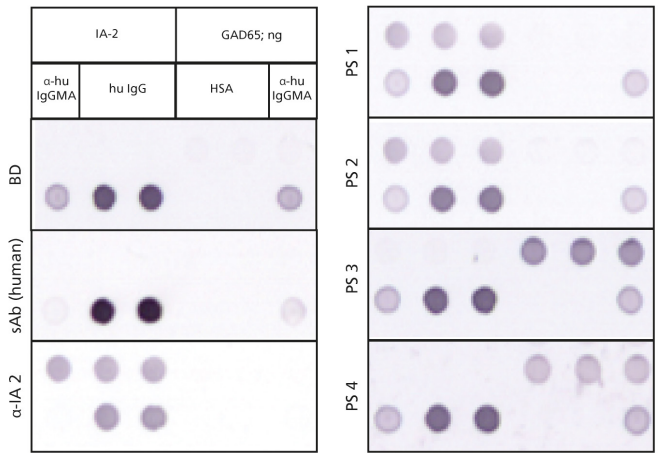
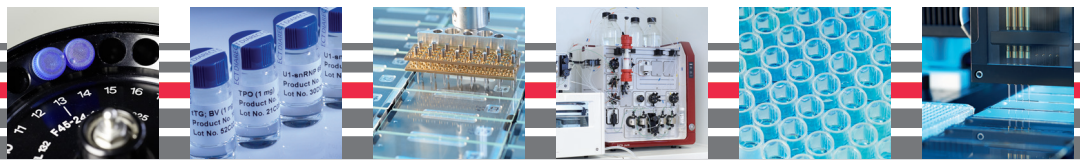


Figure: Immunodot analyses of new generation GAD65; ng and IA-2 in triplicates using a rabbit polyclonal anti-IA-2 antibody (α-IA-2), an anti-human secondary antibody (sAb (human)), sera from T1DM patients (PS1-4) and a blood donor (BD). As positive controls, goat anti-human IgGMA (α-hu IgGMA) and IgG were used. As negative control HSA was spotted on nitrocellulose membrane.

References:
Achenbach *et al.* (2013) Diabetologia. 56 (7): 1615-1622
Bækkeskov *et al.* (1990) Nature. 347 (6289): 151-156
Bonifacio *et al.* (1995) J Immunol. 155 (11): 5419-5426
Bottazzo *et al.* (1974) Lancet. 2 (7892): 1279-1283
Brilliant *et al.* (1990) Genomics. 6 (1): 115-122
Bu *et al.* (1992) PNAS. 89 (6): 2115-2119
Christie *et al.* (1994) Diabetes. 43 (10): 1254-1259
Hagopian *et al.* (1993) Diabetes. 42 (4): 631-636
Lampasona *et al.* (1996) J Immunol. 157 (6): 2707-2711
Landin-Olsson *et al.* (1992) Diabetologia. 35 (11): 1068-1073
Passini *et al.* (1995) PNAS. 92 (20): 9412-9416
Pihoker *et al.* (2005) Diabetes. 54 (2): 52-61
Solimena *et al.* (1994) J Cell Biol. 126 (2): 331-341
Solimena *et al.* (1996) EMBO J. 15 (9): 2102-2114
Winter *et al.* (2002) Clinical Diabetes. 20 (4): 183-191
Winter *et al.* (2011) Clin Chem. 57 (2): 168-175
Ziegler *et al.* (2013) JAMA. 309 (23): 2473-2479



DIARECT GmbH · Bötzinger Str. 29 B · 79111 Freiburg · Germany
Tel. +49 (0) 761 47979-0 · Fax +49 (0) 761 47979-29 · orders-dia@bbisolutions.com · www.bbisolutions.com