



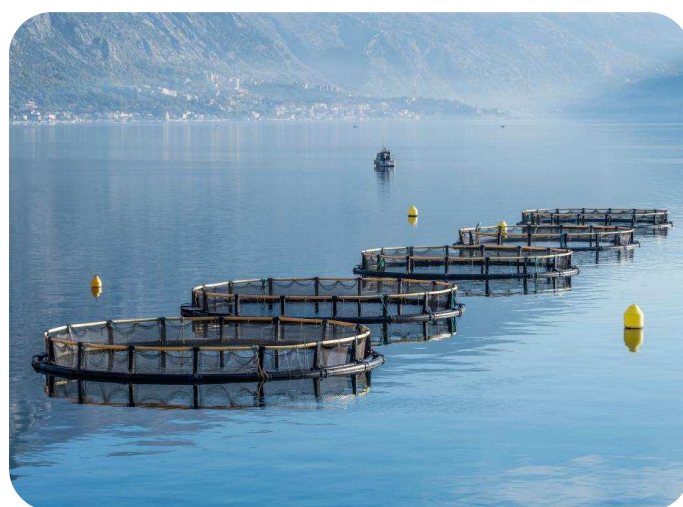
Joint Research Centre

ERM[®]-AD482 and ERM[®]-AD483: Certified Reference Materials to identify the origin of animal proteins in feed

The JRC released two new, certified reference materials (CRMs) for the detection of the animal origin of feed ingredients. The availability of suitable analytical methods and CRMs supports the re-authorisation of certain processed animal proteins as feed ingredient according to Regulation (EU) No 51/2013.

In 2001, a total feed ban for processed animal proteins (PAP) was introduced to tackle the mad cow disease or Bovine Spongiform Encephalopathy (BSE) epidemic. Twelve years later, PAPs derived from non-ruminant farmed animals have been re-authorised in fish feed by Commission Regulation (EU) No 51/2013. Since these PAPs are a valuable source of proteins for feeding stuff, which are a scarce resource, the overall sustainability of farming and the aquaculture sector, in particular, is expected to be boosted.

The European Union Reference Laboratory for Animal Proteins (EURL-AP), hosted by the Centre Wallon de Recherches Agronomiques in Gembloux, Belgium, validated two DNA-based methods for the detection of animal proteins of ruminant or porcine origin. As both methods require calibration, the JRC in Geel provided its expertise and facilities in the development and production of two PAP CRMs: ERM-AD482 for the detection of ruminant material and ERM-AD483 for the detection of porcine materials. These reference materials are certified for ultra-low amounts of species-specific DNA fragments expressed in DNA copy number concentrations and will allow control laboratories to determine their individual cut-off values for presence/absence decisions.



Release of new Certified Reference Materials to identify the origin of animal proteins in feed ©radzonimo - Fotolia.com

ERM-AD482 and ERM-AD483 is a sets of 3 solutions of a double stranded plasmid which contains a specific DNA fragment that is exclusively found in the nucleus of ruminant (ERM-AD482) or porcine (ERM-AD483) animal cells. Each plasmid solution has a certified copy number concentration:

	Copy number concentration of the plasmid	
	Certified value [cp/μL]	Uncertainty [cp/μL]
ERM-AD482a	123	30
ERM-AD482b	32	7
ERM-AD482c	8	3
ERM-AD483a	126	18
ERM-AD483b	34	6
ERM-AD483c	9	3

Details on ERM-AD482 and ERM-AD483

Materials

ERM-AD482 and ERM-AD483 are sets of 3 vials containing solutions of a plasmids exclusively found in the nucleus of ruminant animal cells (ERM-AD482) or in the mitochondria of porcine animal cells (ERM-AD483) at different copy number concentrations. Solutions are made of 1 mmol/L Tris, 0.01 mmol/L EDTA pH 8.0 and supplemented with approximately 12 ng/μL of *Zea mays* DNA (ERM-AD482) or 51 ng/μL of Salmon Sperm, *Oncerhynchus keta*, genomic DNA. Each of the vials contains approximately 1000 μL of plasmid solution.

Homogeneity and stability

The homogeneity, short term and long term stability were demonstrated. Each of the plasmid solutions was found to be homogeneous both within and between vials provided that the minimal sample intake of 4 μL is respected. The plasmid solutions can be safely stored for one year at -20 ± 5 °C and transported on dry-ice. Under the condition that contaminations have been excluded, the solutions can be used for several experiments. The material should however not pass more than 5 (ERM-AD482) or 3 (ERM-AD483) freeze/thaw cycles.

Characterisation

The plasmid solutions of ERM-AD482 and ERM-AD483 are certified for the number of specific DNA fragments per plasmid and the copy number concentration of the plasmid. The certified values for the copy number concentration of the plasmid are based on the results from digital PCR measurements performed by 7 laboratories of demonstrated competence.

Intended use

ERM-AD482 and ERM-AD483 are intended to be used to determine cut-off values as defined in the Standard Operating Procedures, edited by the European Union Reference Laboratory for Animal Proteins in feeding stuffs:

<http://eurl.craw.eu/img/page/sops>

http://eurl.craw.eu/img/page/sops/Cut-off_determination_exact_copy_number_plus_copies_cut-off_V1.0.xls

Each set should be used to construct calibration curves at low copy number concentration to determine a cut-off value. This cut-off value is used in quantitative PCR to discriminate processed animal protein samples which are ruminant/porcine negative from positive samples carrying specific DNA from ruminant/porcine tissue.

To make the plasmid solutions ready for use, the material in the vials have to be thawed completely at room temperature and mixed gently by inverting the vial several times. The vials should be opened and handled in a laminar flow cabinet to reduce the risk of contamination.

Further information

Detailed technical reports are available on <https://crm.irmm.jrc.ec.europa.eu>

How to order

From the JRC in Geel

Tel.: +32 14 571 705 • Fax: +32 14 590 406
<http://www.referencematerials.eu>
E-mail: jrc-rm-distribution@ec.europa.eu

From authorised distributors

LGC Standards GmbH (DE)
<http://www.lgcstandards.com/>

Sigma-Aldrich RTC Inc. (USA)
<http://www.sigmaaldrich.com/irmm>

Sigma-Aldrich Chemie GmbH (CH)
<http://www.sigmaaldrich.com/irmm>

ARMI (USA)
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Accredited CRM Producer: we are accredited to ISO Guide 34:2009 for the production of reference materials under the code BELAC 268-RM

