SUMMARY

(In accordance with 40 CFR part 152, this summary is available for public release after registration)

STUDY TITLE

Transformation Information for Plasmid pDAB8264

DATA REQUIREMENTS

Not Applicable

AUTHOR(S)

Meibao Zhuang, Dayaker Pareddy

STUDY COMPLETED ON

29-June-2011

PERFORMING LABORATORY

Regulatory Sciences and Government Affairs—Indianapolis Lab
Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, Indiana 46268-1054

LABORATORY STUDY ID

101880

Transformation Information for Plasmid pDAB8264

SUMMARY

This report describes the plant transformation method used for plasmid pDAB8264.

STUDY TITLE

Transformation Information for Plasmid pDAB8264

DATA REQUIREMENTS

Not Applicable

AUTHOR(S)

Meibao Zhuang (317)-337-3689
[mZhuang@dow.com]
Dayaker Pareddy

STUDY COMPLETED ON

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PERFORMING LABORATORY

Regulatory Sciences and Government Affairs—Indianapolis Lab
Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, Indiana 46268-1054

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Compound: pDAB8264

Title: Transformation Information for Plasmid pDAB8264

- STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS:

  No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec. 10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA sec. 10(g).

Company: Dow AgroSciences LLC

Company Agent: Mark Krieger

Title: Regulatory Manager

Signature: 

Date: 23 Feb 2011

THIS DATA MAY BE CONSIDERED CONFIDENTIAL IN COUNTRIES OUTSIDE THE UNITED STATES.

STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Title: Transformation Information for Plasmid pDAB8264

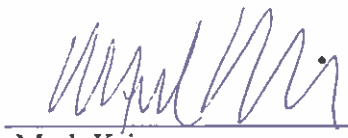
Study Initiation Date: 10/09/2010

This report represents data generated after the effective date of the EPA FIFRA Good Laboratory Practice Standards.

United States Environmental Protection Agency
Title 40 Code of Federal Regulations Part 160
FEDERAL REGISTER, August 17, 1989

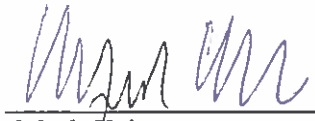
Organisation for Economic Co-Operation and Development
ENV/MC/CHEM(98)17, Paris January 26, 1998

This study does not meet requirements of 40 CFR Part 160.



23 Feb 2011

Mark Krieger
Sponsor
Dow AgroSciences LLC — Date



23 Feb 2011

Mark Krieger
Submitter
Dow AgroSciences LLC — Date

29 - June - 2011

Meibao Zhuang
Study Director/Author
Dow AgroSciences LLC — Study Completion Date

QUALITY ASSURANCE STATEMENT

Compound: pDAB8264

Title: Transformation Information for Plasmid pDAB8264

Study Initiation Date: 10/09/2010 Study Completion Date:

NON-GLP STUDY

SIGNATURE PAGE

29-June-2011

Meibao Zhuang
Author
Dow AgroSciences LLC

Date

6/27/2011

Dayakar Pareddy
Co-Author
Dow AgroSciences LLC

Date

27-June-2011

Ping Song
Peer Reviewer
Dow AgroSciences LLC

Date

27 JUNE 2011

Guomin Shan
Science Leader
Dow AgroSciences LLC

Date

27 JUN 2011

Kathryn A. Clayton
BRS Global Leader
Dow AgroSciences LLC

Date

STUDY PERSONNEL

Title: Transformation Information for Plasmid pDAB8264

Principal Analyst: Meibao Zhuang
(Principle Investigator)

Analysts: Dayakar Pareddy

TABLE OF CONTENTS

Transformation Information for Plasmid pDAB8264

ABSTRACT

This report describes the plant transformation method used for plasmid pDAB8264.

TRANSFORMATION SYSTEM

Transgenic soybean (*Glycine max*) was generated through *Agrobacterium*-mediated transformation of soybean cotyledonary node explants. The disarmed *Agrobacterium* strain EHA101 (Hood, Helmer et al. 1986), carrying the binary vector with the selectable marker (*pat*) and the genes of interest (*aad-12* and *2mEPSPS v1*) within the T-DNA region, was used to initiate transformation.

Agrobacterium-mediated transformation was carried out using a modified procedure of Zeng et al. (Zeng, Vadnais et al. 2004). Briefly, soybean seeds (cv Maverick) were germinated on basal media and cotyledonary nodes were isolated and infected with *Agrobacterium.* Shoot initiation, shoot elongation, and rooting media were supplemented with cefotaxime, timentin and vancomycin for removal of *Agrobacterium*. Glufosinate selection was employed to inhibit the growth of non-transformed shoots. Selected shoots were transferred to rooting medium for root development and then transferred to soil mix for acclimatization of plantlets.

Terminal leaflets of selected plantlets were leaf painted with glufosinate to screen for putative transformants. The screened plantlets were transferred to the greenhouse, allowed to acclimate and then painted with glufosinate to reconfirm tolerance and deemed to be putative transformants. The screened plants were sampled and molecular analyses for the confirmation of the selectable marker gene and/or the genes of interest were carried out. Specifically, for T0 plants, PCR analysis was performed to verify the absence of the spectinomycin backbone region as well as the presence of the *aad*-12 coding region and *2mEPSPS* PTU. Invader assay was conducted for copy number detection for *pat*, *aad*-12, and *2mEPSPS* genes. Selected T0 plants were allowed to self-fertilize in the greenhouse to give rise to T1 seed. For T1 plants, PCR analysis, Invader assay, and Southern blot analysis were performed to detect copy number, integration number, and PTU integrity.

REFERENCE

Hood, E. E., G. L. Helmer, et al. (1986). "The Hypervirulence of *Agrobacterium tumefaciens* A281 Is Encoded in a Region of pTiBo542 Outside of T-DNA." Journal of Bacteriology **168**(3): 1291-1301.

Zeng, P., D. A. Vadnais, et al. (2004). "Refined glufosinate selection in Agrobacterium-mediated transformation of soybean [Glycine max (L.) Merrill]." Plant Cell Reports **22**(7): 478-482.