



**BUDAPEST RESTRICTED CERTIFICATE OF DEPOSIT
BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MATERIAL FOR THE PURPOSES OF PATENT PROCEDURE
INTERNATIONAL FORM
RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2**

The American Type Culture Collection (ATCC®) has received your deposit of seeds/strain(s)/strain(s) in connection with the filing of an application for patent. The following information is provided to fulfill Patent Office requirements.

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Deposited on Behalf of: **Singapore Health Services Pte Ltd**

Date of Receipt of seeds/strain(s) by the ATCC®: **October 12, 2017**

<u>Identification Reference by Depositor:</u>	<u>ATCC ®Patent Deposit Designation:</u>	<u>Quantity Received:</u>
Mouse Hybridoma cells; 13E4-8	PTA-124525	26 vials
Mouse Hybridoma cells; 4H5-10	PTA-124526	26 vials
Mouse Hybridoma cells; 7B9-6	PTA-124527	26 vials
Mouse Hybridoma cells; 10C8-11	PTA-124528	26 vials
Mouse Hybridoma cells; 3G11-2	PTA-124529	26 vials
Mouse Hybridoma cells; 4H2-3	PTA-124530	26 vials

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The deposit was tested **October 31, 2017** and on that date, the seeds/strain(s) were viable.
International Depository Authority: American Type Culture Collection (ATCC®), Manassas, VA, USA.

Signature of person having authority to represent ATCC®:

**Michiyo
Gilpin**

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November 13, 2017
Date

cc: Richard CLEGG

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polypeptide of interest. The methods comprise fusing a cell capable of producing one more antibodies capable of binding to the mutant p53 polypeptide of interest isolated from the animal with a myeloma cell, to produce a hybridoma.

- 5 In particular, the methods of the present invention are for producing monoclonal antibodies capable of binding to a mutant p53 polypeptide. This is achieved by immunizing an animal with immunogen according to the invention, and subsequently generating monoclonal hybridomas from cells isolated from the animals, the hybridomas producing antibodies of a single type (i.e. of a single specificity), which are capable of binding to the mutant p53
10 polypeptide.

Methods for hybridoma formation are well known to the skilled person, and are described, for example, in *Antibodies: A Laboratory Manual*, Second Edition, 2014; Edward A. Greenfield, Cold Spring Harbor Laboratory Press (incorporated by reference herein above); in particular
15 at Chapter 7. Briefly, an animal is immunized in accordance with the invention, stimulating an adaptive immune response, and B lymphocytes are isolated from the animal and fused with a suitable myeloma cell line to produce a hybridoma. B lymphocytes and myeloma cells may be fused using methods known to the skilled person. For example, cells may be fused by co-centrifugation in polyethylene glycol (PEG). Following fusion, cells are plated-out by
20 limiting dilution in tissue culture plates to roughly a single cell per well, and cultured *in vitro*. Hybridomas may be selected by culture using selective media known in the art (e.g. media containing hypoxanthine-aminopterin-thymidine (HAT)), which unfused lymphocytes and myeloma cells are unable to grow or survive in.

- 25 Antibodies produced by the methods of the invention may be produced on a large scale using methods known to the skilled person. Hybridomas can be propagated either in *in vitro* culture using standard methods of cell culture, or *in vivo*, e.g. as ascites in a host animal. In some embodiments, the methods of the present invention comprise propagating the hybridoma by *in vitro* cell culture. In some embodiments, the methods comprise propagating
30 the hybridoma *in vivo* by injecting a host animal with the hybridoma.

~~Antibodies according to the invention can also be produced recombinantly, as described hereinbelow. For example, a polynucleotide encoding a monoclonal antibody can be isolated from a B cell or hybridoma cell producing an antibody, e.g., by reverse transcription PCR
35 (RT-PCR) using oligonucleotide primers that specifically amplify the genes encoding the heavy and light chains of the antibody, and the sequence of the polynucleotide can be~~

Hybridoma 13E4 ("Mouse hybridoma cells 13E4-8") was deposited by Singapore Health Services Pte Ltd on 12 October 2017 at the American Type Culture Collection (ATCC®), Manassas, VA, USA under accession number PTA-124525 in accordance with the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure (herein referred to as the 'Budapest Treaty').

Hybridoma 4H5 ("Mouse Hybridoma cells; 4H5-10") was deposited by Singapore Health Services Pte Ltd on 12 October 2017 at the American Type Culture Collection (ATCC®), Manassas, VA, USA under accession number PTA-124526 in accordance with the provisions of the Budapest Treaty.

Hybridoma 7B9 ("Mouse Hybridoma cells; 7B9-6") was deposited by Singapore Health Services Pte Ltd on 12 October 2017 at the American Type Culture Collection (ATCC®), Manassas, VA, USA under accession number PTA-124527 in accordance with the provisions of the Budapest Treaty.

Hybridoma 10C8 ("Mouse Hybridoma cells; 10C8-11") was deposited by Singapore Health Services Pte Ltd on 12 October 2017 at the American Type Culture Collection (ATCC®), Manassas, VA, USA under accession number PTA-124528 in accordance with the provisions of the Budapest Treaty.

Hybridoma 3G11 ("Mouse Hybridoma cells; 3G11-2") was deposited by Singapore Health Services Pte Ltd on 12 October 2017 at the American Type Culture Collection (ATCC®), Manassas, VA, USA under accession number PTA-124529 in accordance with the provisions of the Budapest Treaty.

Hybridoma 4H2 ("Mouse Hybridoma cells; 4H2-3") was deposited by Singapore Health Services Pte Ltd on 12 October 2017 at the American Type Culture Collection (ATCC®), Manassas, VA, USA under accession number PTA-124530 in accordance with the provisions of the Budapest Treaty.

Antibodies according to the invention can also be produced recombinantly, as described hereinbelow. For example, a polynucleotide encoding a monoclonal antibody can be isolated from a B cell or hybridoma cell producing an antibody, e.g., by reverse transcription PCR (RT-PCR) using oligonucleotide primers that specifically amplify the genes encoding the heavy and light chains of the antibody, and the sequence of the polynucleotide can be