CLAIMS

1. Monoclonal rabbit antibody, or a fragment thereof, against integrin with insect-derived glycosylation pattern and integrin with another eukaryotic glycosylation pattern, each comprising at least a light chain variable region (V_L) and a heavy chain variable region (V_H), wherein the antibody has antigen binding specificity for a non-occluded epitope of an extracellular integrin domain or extracellular integrin chain domain, and wherein the antibody is able to bind to intact heterodimers of integrin in formalin fixed paraffin embedded (FFPE) material and on viable cells with the substantially same specificity.

2. Antibody according to claim 1, wherein the antibody binds to the extracellular domain of integrin αvß3 and preferably, V_L comprises an amino acid sequence of SEQ ID NO: 95 (V_L-αvß3) and V_H comprises an amino acid sequence of SEQ ID NO: 96 (V_H-αvß3).

3. Antibody according to claim 1, wherein the antibody binds to the extracellular domain of integrin αvß5 and preferably, V_L comprises an amino acid sequence of SEQ ID NO: 15 (V_L-αvß5) and V_H comprises an amino acid sequence of SEQ ID NO: 16 (V_H-αvß5).

4. Antibody according to claim 1, wherein the antibody binds to the extracellular domain of integrin αvß6 and preferably, V_L comprises an amino acid sequence of SEQ ID NO: 135 (V_L-αvß6) and V_H comprises an amino acid sequence of SEQ ID NO: 136 (V_H-αvß6).

5. Antibody according to claim 1, wherein the antibody binds to the extracellular domain of integrin αvß8 and preferably, V_L comprises an amino acid sequence of SEQ ID NO: 175 (V_L-αvß8) and V_H comprises an amino acid sequence of SEQ ID NO: 176 (V_H-αvß8).

6. Antibody according to claim 1, wherein the antibody binds to the extracellular domain of integrin chain αv and preferably, V_L comprises an amino acid sequence of SEQ ID NO: 215 (V_L-αv) and V_H comprises an amino acid sequence of SEQ ID NO: 216 (V_H-αv).

7. Polynucleotide encoding the antibody, or a fragment thereof, of any of claims 1 to 6.

8. Recombinant immunogen consisting of an extracellular integrin domain with insect-derived glycosylation pattern, optionally coupled as delta-trans membrane form.

9. Immunogen according to claim 9 having an amino acid sequence of SEQ ID NOs: 10, 90, 130, 170 or 210, or variants, mutants, parts of the amino acid sequence or at least 95 % homologous sequences having the same function.

10. Polynucleotide encoding the immunogen of claim 9.

11. Monoclonal antibodies obtained by immunizing a rabbit with the immunogen according to claim 8 or 9 and/or the polynucleotide according to claim 10, taking polyclonal antiserum with polyclonal antibodies and preparing the monoclonal antibodies.

12. Method for preparing monoclonal rabbit antibodies comprising the steps of:
(a) recombinantly expressing an extracellular integrin domain in insect cells,
(b) purifying the expressed extracellular integrin domain,
(c) immunizing a rabbit with the purified extracellular integrin domain,
(d) taking polyclonal antiserum comprising polyclonal antibodies from the rabbit, and
(e) preparing the monoclonal antibodies.

13. Method for manufacturing a recombinant monoclonal antibody comprising a light chain variable region (V_L) and a heavy chain variable region (V_H) with the steps of:
(a) introducing at least one vector comprising antibody-encoding nucleic acid sequences of
(i) SEQ ID NO: 115 (V_L-αvß3) and SEQ ID NO: 116 (V_H-αvß3),
(ii) SEQ ID NO: 35 (V_L-αvß5) and SEQ ID NO: 36 (V_H-αvß5),
(iii) SEQ ID NO: 155 (V_L-αvß6) and SEQ ID NO: 156 (V_H-αvß6),
(iv) SEQ ID NO: 195 (V_L-αvß8) and SEQ ID NO: 196 (V_H-αvß8), or
(v) SEQ ID NO: 235 (V_L-αv) and SEQ ID NO: 236 (V_H-αv)
into a host cell,
(b) cultivating the host cell in a culture medium, thereby expressing the encoded antibody, and
(c) purifying the expressed antibody.

14. Use of the antibody, or a fragment thereof, of any of claims 1 to 6 or 11 for the detection of integrins in formalin fixed paraffin embedded (FFPE) material.

15. Method for screening anti-integrin antibodies, which are capable of discriminating between the respective closest homologues of integrin α-subunit and/or ß-subunit and suitable for immunohistochemistry in formalin fixed paraffin embedded (FFPE) material, comprising the steps of:

(a) providing a sample of antibodies being capable of binding a selected integrin,

(b) aligning integrin sequences to identify the closest homologue of the α-subunit and/or ß-subunit of the selected integrin,

(c) performing a differential ELISA on native forms of the selected integrin and the closest homologue(s) thereof with the antibody sample, thereby accumulating antibodies against the selected integrin (primary screen),

(d) performing another differential ELISA on native forms of the selected integrin and another integrin with the accumulated antibodies of step (c), thereby further accumulating antibodies against the selected integrin (secondary screen),

(e) performing immunohistochemistry of FFPE cell lines with the accumulated antibodies of step (d), wherein at least one cell line is capable of expressing the selected integrin and optionally another cell line is not capable of expressing the selected integrin, thereby further accumulating antibodies against the selected integrin (tertiary screen),

(f) performing immunohistochemistry of FFPE cell lines of step (e) with the accumulated antibodies of step (e), wherein the cell line is grown as xenograft tumor in a mammal, thereby further accumulating antibodies against the selected integrin (quaternary screen), and

(g) performing immunohistochemistry of archival FFPE tumors with the accumulated antibodies of step (f), thereby further accumulating antibodies against the selected integrin (quintenary screen).

We claim:

1. A monoclonal rabbit antibody, or antigen-binding fragment thereof, against integrin with insect-derived glycosylation pattern and/or integrin with another eukaryotic glycosylation pattern, each comprising at least a light chain variable region (VL) and a heavy chain variable region (VH), wherein the antibody has antigen binding specificity for an exposed epitope of an extracellular integrin domain or extracellular integrin chain domain, and wherein the antibody is able to bind to intact heterodimers of integrin in formalin fixed paraffin embedded (FFPE) material and on viable cells with the substantially same specificity wherein said antibody is selected from the group consisting of: an antibody that binds to the extracellular domain of integrin $\alpha v\beta 3$ and V_L comprises the amino acid sequence of SEQ ID NO: 95 (V_L-$\alpha v\beta 3$) and V_H comprises the amino acid sequence of SEQ ID NO: 96 (V_H-$\alpha v\beta 3$); an antibody that binds to the extracellular domain of integrin $\alpha v\beta 5$ and V_L comprises the amino acid sequence of SEQ ID NO: 15 (V_L-$\alpha v\beta 5$) and V_H comprises the amino acid sequence of SEQ ID NO: 16 (V_H-$\alpha v\beta 5$); an antibody that binds to the extracellular domain of integrin $\alpha v\beta 6$ and V_L comprises the amino acid sequence of SEQ ID NO: 135 (V_L-$\alpha v\beta 6$) and V_H comprises the amino acid sequence of SEQ ID NO: 136 (V_H-$\alpha v\beta 6$); an antibody that binds to the extracellular domain of integrin $\alpha v\beta 8$ and V_L comprises the amino acid sequence of SEQ ID NO: 175 (V_L-$\alpha v\beta 8$) and V_H comprises the amino acid sequence of SEQ ID NO: 176 (V_H-$\alpha v\beta 8$); and an antibody that binds to the extracellular domain of integrin chain αv and V_L comprises the amino acid sequence of SEQ ID NO: 215 (V_L-αv) and V_H comprises the amino acid sequence of SEQ ID NO: 216 (V_H-αv).

2. A polynucleotide which encodes the antibody, or antigen-binding fragment thereof, of claim 1.

3. A method for obtaining the monoclonal antibody according to claim 1, comprising immunizing a rabbit with an immunogen which comprises an extracellular integrin domain with insect-derived glycosylation pattern, optionally coupled as delta-trans membrane form; obtaining a polyclonal antiserum comprising polyclonal antibodies from said rabbit; and preparing the monoclonal antibodies.

4. A method for manufacturing a recombinant monoclonal antibody comprising a light chain variable region (V_L) and a heavy chain variable region (V_H) with the steps of: (a) introducing at least one vector comprising antibody-encoding nucleic acid sequences of (i) SEQ ID NO: 115 (V_L-$\alpha v\beta 3$) and SEQ ID NO: 116 (V_H-$\alpha v\beta 3$), (ii) SEQ ID NO: 35 (V_L-$\alpha v\beta 5$) and SEQ ID NO: 36 (V_H-$\alpha v\beta 5$), (iii) SEQ ID NO: 155 (V_L-$\alpha v\beta 6$) and SEQ ID NO: 156 (V_H-$\alpha v\beta 6$), (iv) SEQ ID NO: 195 (V_L-$\alpha v\beta 8$) and SEQ ID NO: 196 (V_H-$\alpha v\beta 8$), or (v) SEQ ID NO: 235 (V_L-αv) and SEQ ID NO: 236 (V_H-αv) into a host cell, (b) cultivating the host cell in a culture medium, thereby expressing the encoded antibody, and (c) purifying the expressed antibody.

5. A method for the detection of integrins in formalin fixed paraffin embedded (FFPE) material, comprising contacting said FFPE material with the antibody or an antigen binding fragment thereof of claim 1.