QUANTITATIVE DETERMINATION OF HUMAN PYRUVATE KINASE M2

Human Pyruvate Kinase M2 ELISA

- High sensitivity (0.15 ng/ml)
- Excellent analytical characteristics
- Validated for human serum, plasma, urine and stool samples
Introduction

Pyruvate kinase isoenzyme type M2 (PKM2, also called PK2, Thyroid-hormone binding protein, and Tumor M2-PK) is a phosphotyrosine-binding protein, as evidenced by the observation that nuclear PKM2 binds to tyrosine-phosphorylated-catenin and activates catenin [5].

PKM2 is one of four pyruvate kinase isoenzymes which differ widely in their occurrence according to the type of tissue, their kinetic characteristics and regulation mechanism [3, 10]. PKM2 may exist in both, tetrameric and dimeric forms. Each monomer of PKM2 consists of 531 amino acids and can be subdivided into four domains: the N-domain, the A-domain, the B-domain and the C-domain [10]. The molecular weight of the PKM2 monomer is 58 kD. PKM2 is encoded by the PKM gene and is the product of 2 mutually exclusive alternatively spliced exons (exon 9 and 10) [7].

Pyruvate kinase isoenzyme type M2 is expressed in some differentiated tissues, such as lung, fat tissue, retina, pancreatic islets as well as in all cells with a high rate of nucleic acid synthesis, which include all proliferating cells, such as normal proliferating cells, embryonic cells, adult stem cells and especially tumor cells [5,7].

Immunohistological staining of Tumor M2-PK in various rat and human tumors (breast, renal, lung, colon, rectal and skin tumors) revealed that increased Tumor M2-PK in tumor cells is a general metabolic alteration during tumorigenesis and correlated with malignancies of the tumors [11].

Tumor M2-PK levels are increased in EDTA-plasma samples from patients with solid tumors at various sites, including renal, lung, breast cancers, renal cell carcinoma and testicular cancer.

Elevation of serum PKM2 levels was reported in patients with colon cancer, breast cancer, urological tumors, lung carcinoma, cervical cancer and gastrointestinal tumor [4].

Recent studies describe the clinical utility of the determination of Tumor M2-PK in the patients with colorectal cancer (CRC).

Regarding serum biomarkers, due to the heterogenous nature of CRC, a single biomarker is unlikely to have sufficient sensitivity or specificity for use as a stand-alone diagnostic screening test and panel of markers may be more effective. A three biomarker panel was identified that has high sensitivity and specificity for early stage of CRC. This model consisting of Dickkopf-3 (DKK3), Insulin like growth factor binding protein 2 (IGFBP2) and Pyruvate kinase M2 (PKM2), raising the possibility for its use as non-invasive blood diagnostic or screening test [1].
Tumor M2-PK was measured in the feces of patients with colonoscopy-proven cancer of the colon and rectum. The feecal levels of Tumor M2-PK are significantly higher in patients with colorectal cancer than in the control group and determination of Tumor M2-PK in stool samples might be also a valuable new screening tool for colorectal cancer [11].

Pyruvate kinase has been recognized as an attractive target for cancer therapy. In its metabolic role as terminal enzyme of glycolysis, its activity determines cellular energy level, redox homeostasis and ability to proliferate.

Pyruvate kinase also regulates the final rate-limiting step of glycolysis and catalyzes the transfer of a phosphate group from phosphoenolpyruvate (PEP) to adenosine diphosphate [5, 9].

Tyrosine kinases may also be responsible for the Warburg effect in cancer, as they can phosphorylate glycolytic enzymes, including PKM2, and then promote tumor growth [3].

### Intended use

The RD191345200R Human Pyruvate Kinase M2 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human pyruvate kinase M2.

- It is intended for research use only
- The total assay time is less than 3.5 hours
- The kit measures decorin in serum and urine samples
- Assay format is 96 wells
- Standard is recombinant protein based
- Components of the kit are provided ready to use, concentrated or lyophilized

### Clinical application

- Oncology

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<table>
<thead>
<tr>
<th>HUMAN PYRUVATE KINASE M2 ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT. NO.: RD191345200R</td>
</tr>
</tbody>
</table>

- **Assay format**: Sandwich ELISA, Biotin-labelled antibody, 96 wells/kit
- **Samples**: Serum, plasma, stool, urine
- **Standards**: 0.5 to 32 ng/ml
- **Limit of detection**: 0.15 ng/ml

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**Test principle**

In the BioVendor Human Pyruvate Kinase M2 ELISA, standards and samples are incubated in microplate wells pre-coated with polyclonal anti-human pyruvate kinase M2 antibody. After 60 minutes incubation at RT and washing, biotin labelled sheep polyclonal anti-human decorin antibody is added and incubated at RT for 60 minutes with captured pyruvate kinase M2. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation at RT and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of pyruvate kinase M2. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.
**Precision**

Intra-assay (Within-Run) (n=8)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (ng/ml)</th>
<th>SD (ng/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>22.47</td>
<td>1.17</td>
<td>5.2</td>
</tr>
<tr>
<td>Serum 2</td>
<td>8.30</td>
<td>0.47</td>
<td>5.7</td>
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</table>

Inter-assay (Run-to-Run) (n=6)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (ng/ml)</th>
<th>SD (ng/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>7.98</td>
<td>0.60</td>
<td>7.3</td>
</tr>
<tr>
<td>Serum 2</td>
<td>26.40</td>
<td>0.99</td>
<td>3.7</td>
</tr>
</tbody>
</table>

**Spiking recovery**

Samples were spiked with different amounts of human pyruvate kinase M2 and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Observed (ng/ml)</th>
<th>Expected (ng/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>10.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>27.72</td>
<td>34.02</td>
<td>81.5</td>
</tr>
<tr>
<td></td>
<td>18.31</td>
<td>22.02</td>
<td>83.1</td>
</tr>
<tr>
<td></td>
<td>14.09</td>
<td>16.02</td>
<td>87.9</td>
</tr>
<tr>
<td>Serum 2</td>
<td>11.78</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>26.96</td>
<td>35.78</td>
<td>75.3</td>
</tr>
<tr>
<td></td>
<td>18.63</td>
<td>23.78</td>
<td>78.3</td>
</tr>
<tr>
<td></td>
<td>15.46</td>
<td>17.78</td>
<td>86.9</td>
</tr>
</tbody>
</table>

**Linearity**

Serum and plasma samples were serially diluted with Dilution Buffer and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Observed (ng/ml)</th>
<th>Expected (ng/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>2x</td>
<td>16.07</td>
<td>15.33</td>
<td>104.8</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>8.60</td>
<td>7.67</td>
<td>112.2</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>4.33</td>
<td>3.83</td>
<td>112.9</td>
</tr>
<tr>
<td>Serum 2</td>
<td>2x</td>
<td>12.98</td>
<td>11.09</td>
<td>117.1</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>6.43</td>
<td>5.54</td>
<td>116.0</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>3.13</td>
<td>2.77</td>
<td>112.9</td>
</tr>
</tbody>
</table>

**Summary of protocol**

- Reconstitute Master Standard and prepare set of Standards
- Dilute samples
- Add 100 µl Standards and samples
- Incubate at RT/rpm for 1 hour without shaking
- Wash plate 3 times
- Add 100 µl Biotin Labelled Antibody
- Incubate at RT/rpm for 1 hour without shaking
- Wash plate 3 times
- Add 100 µl Streptavidin-HRP Conjugate
- Incubate at RT/rpm for 30 minutes without shaking
- Wash plate 3 times
- Add 100 µl Substrate Solution
- Incubate at RT for 10 min
- Add 100 µl stop solution
- Read absorbance and calculate results
Preliminary Population Data

The following results were obtained when serum samples from 166 unselected donors (94 men + 72 women) 21-65 years old were assayed with the Biovendor Human Pyruvate Kinase M2 ELISA in our laboratory.

### Age and Sex Dependent Distribution of Pyruvate Kinase M2

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>n</th>
<th>Mean PKM2 (ng/ml)</th>
<th>Median PKM2 (ng/ml)</th>
<th>SD PKM2 (ng/ml)</th>
<th>Min. PKM2 (ng/ml)</th>
<th>Max. PKM2 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>21-29</td>
<td>18</td>
<td>19.09</td>
<td>18.51</td>
<td>5.33</td>
<td>12.38</td>
<td>32.56</td>
</tr>
<tr>
<td></td>
<td>30-39</td>
<td>28</td>
<td>17.66</td>
<td>16.14</td>
<td>6.03</td>
<td>9.61</td>
<td>34.52</td>
</tr>
<tr>
<td></td>
<td>40-49</td>
<td>32</td>
<td>17.99</td>
<td>16.40</td>
<td>7.18</td>
<td>7.79</td>
<td>40.63</td>
</tr>
<tr>
<td></td>
<td>50-65</td>
<td>17</td>
<td>14.89</td>
<td>14.20</td>
<td>3.71</td>
<td>9.79</td>
<td>24.73</td>
</tr>
<tr>
<td>Women</td>
<td>22-29</td>
<td>13</td>
<td>14.87</td>
<td>15.80</td>
<td>3.71</td>
<td>8.20</td>
<td>23.17</td>
</tr>
<tr>
<td></td>
<td>30-39</td>
<td>28</td>
<td>13.84</td>
<td>13.07</td>
<td>4.01</td>
<td>5.96</td>
<td>20.86</td>
</tr>
<tr>
<td></td>
<td>50-61</td>
<td>9</td>
<td>13.30</td>
<td>13.95</td>
<td>2.82</td>
<td>8.65</td>
<td>16.76</td>
</tr>
</tbody>
</table>
References

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