- adding ~~the~~an anti-(ox)MIF antibody to be determined to a second part of the device, which is configured to be in a connection with the first part of the device which allows cell migration and diffusion, and
- calculating inhibition of migration, and
- wherein no (ox) MIF is added to the device.

In one aspect, there is provided an assay kit for determining the potency of anti-(ox)MIF-antibodies, comprising all reagents necessary to carry out the assay method as defined herein, preferably

- cells which express MIF
- antibody dilution buffer (e.g. glycine buffer)
- migration medium, and/or
- Two chamber cell migration system, wherein the kit comprises no (ox)MIF.

In one ~~aspect~~embodiment, there is provided a pharmaceutical or diagnostic composition, comprising anti-(ox)MIF antibodies, wherein the anti-(ox)MIF antibodies are characterized in that they have undergone a potency determination, as defined herein.

"Potency" in this context is a measure of drug activity expressed in terms of the amount required to produce an effect of given intensity. It is preferably expressed as an IC_{50} value (half maximal inhibition).

The assay of the present disclosure is based on the principle of inhibiting autocrine chemotactic actions of (ox)MIF and thereby inhibiting migration of cells which express MIF, in particular oxMIF, preferably on their surface, e.g. certain monocytic cells, like U937 monocytic cells. The cells of the present disclosure can express MIF endogenously or can be manipulated to recombinantly express MIF. The MIF needs to be expressed as oxMIF/converted to oxMIF. In a preferred embodiment the oxMIF is expressed on the surface of the cells, though this is not necessarily a key feature of this disclosure.

The present assay is to be differentiated from a typical chemotaxis assay (as referred to in scientific literature) which is based on a directed migration of cells towards a chemoattractant gradient. This chemoattractant gradient according to the prior art would have been (ox)MIF, added to the device in addition to the cells. The present inventors could surprisingly show that it is possible to provide an assay using cells which express MIF and that it was thus possible to provide an assay without addition of a further chemoattractant, i.e. without addition of (ox) MIF. These findings were particularly advantageous as the resultant assay is ox-MIF-diffusion independent, i.e. diffusion over time will not interfere with the results, and thus the assay is not as time-sensitive as prior art assays. Anti-(ox)MIF antibodies are capable of inhibiting the pro-migratory (ox)MIF functions on cells expressing (ox)MIF, thereby inhibiting random cell migration ("chemokinesis") (rather than inhibiting cell chemotaxis as referred to in scientific literature).

The present disclosure is also further explained by the attached figures.

Figure 1: General set-up of the present assay method.

Figure 2: FACS data of oxMIF on U937 monocytic cells

Figure 3: Exemplary figure showing two migration inhibition curves of U937 (human monocytes) and NR8383 (rat macrophages) towards different concentrations of RAM9 (anti-oxMIF antibody; logarithmic scale). FACS plot shows the binding of RAM9 to the cellular surface of these cells (black line; thin line: isotype control antibody)

CLAIMS

1. Assay method for determining the potency of anti-MIF antibodies, wherein a cell migration is performed, wherein the assay method comprises the following steps:
   - providing cells which are capable of migration in a first part of a device, wherein the cells comprise MIF,
   - adding ~~thean~~ anti-(ox)MIF antibody to be determined, to a second part of the device, which is configured to be in a connection with the first part of the device which allows cell migration and diffusion, and
   - calculating inhibition of migration , and
   - wherein no (ox) MIF is added to the device.
2. Assay method according to claim 1, wherein the anti-MIF antibodies are anti-oxMIF antibodies~~, preferably wherein the antibodies are~~ selected from the group consisting of RAB0 (as identified by SEQ ID NOs:3 and 7), RAB4 (as identified by SEQ ID NOs:2 and 6), RAB9 (as identified by SEQ ID NOs:1 and 5), RAM0 (as identified by SEQ ID NOs:9 and 10), RAM4 (as identified by SEQ ID NOs:13 and 14) and~~/or~~ RAM9 (as identified by SEQ ID NOs:11 and 12), very preferred RAM9.
3. Assay method according to claim 1 or 2, wherein the device comprises an upper and a lower chamber, which are connected via a separating membrane with pores and in that the cells are added to the upper chamber and in that the antibodies are added to the lower chamber, preferably wherein the device is a two chamber cell migration assay (modified Boyden chamber assay), more preferably a Transwell cell migration assay device.
4. Assay method according to any one of the preceding claims, wherein the cells are cells which express (ox)MIF, preferably on their cell surface, preferably wherein the cells are cells from disease samples, more preferred wherein the cells are monocytic cells, preferably human monocytic cells, more preferred human immortalized monocytic cells, most preferred U937 cells, or THP-1 human monocytic cells or in an alternative embodiment rat NR 8383 monocytic cells.
5. Assay method according to any one of the preceding claims, wherein the cells are provided as a cell suspension in a suitable migration medium to allow migration.
6. Assay method according to claim 5, wherein the migration medium does not comprise proteins.
7. Assay method according to any one of the preceding claims, wherein the antibody is added in a non toxic biological buffer with a moderately weak acid and its conjugate base, preferably a glycine buffer, an N-substituted taurine buffer or a phosphate buffered saline (PBS) buffer, to the second part, e.g. lower chamber, of e.g. the Transwell® chamber.
8. Assay method according to any one of the preceding claims, wherein the antibody is added to have a final concentration in the assay of 0.01 – 100 nM, preferably 0.02 to 50 nM, preferably 0.04-30 nM, preferably as a dilution series
9. Assay method according to any one of the preceding claims, wherein the assay, e.g. the Transwell® chamber, is incubated after addition of the cells and the antibody for 6-20 h, preferably 8-12 h, preferably at approximately 37°C, wherein the cells are U937 cells, and wherein the membrane has a pore size of approximately 5 µm.
10. Assay method according to any one of the preceding claims, wherein the migrated cells are counted, preferably after the above incubation step, and preferably in the lower chamber, whereupon

information about the potency of the tested antibody can be obtained, preferably by calculating the half-maximal inhibiting antibody concentration (IC_{50}-value).

11. Assay method according to any one of the preceding claims, wherein the cells undergo from 10 to 48 h, preferably 8-16, preferably 10-12 h serum starvation before they are used in the assay.

~~12. Assay method according to any one of the preceding claims, wherein the cells are derived from a working bank.~~

~~13. Assay method according to any one of the preceding claims, wherein no (ox)MIF is added to the device.~~

142. Assay kit for determining the potency of anti-(ox)MIF-antibodies, comprising all reagents necessary to carry out the assay method of any one or more of the preceding claims, preferably

- cells which express MIF
- antibody dilution buffer (e.g. glycine buffer)
- migration medium, and/or
- Two chamber cell migration system, wherein the kit comprises no (ox)MIF.

~~15. Pharmaceutical or diagnostic composition, comprising anti-(ox)MIF antibodies, wherein the anti-(ox)MIF antibodies are characterized in that they have undergone a potency determination, as defined in any one of the precedings claims.~~